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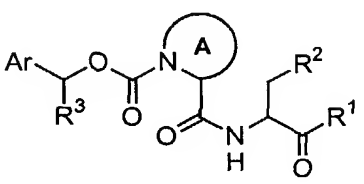
(54) Title: CASPASE INHIBITORS AND USES THEREOF



(I)

(57) Abstract: This invention provides caspase inhibitors having the formula: (I) wherein Ring A is an optionally substituted piperidine, tetrahydroquinoline or tetrahydroisoquinoline ring; R^1 is hydrogen, CHN_2, R, or $-CH_2Y$; R is an optionally substituted group selected from an aliphatic group, an aryl group, an aralkyl group, a heterocyclic group, or an heterocyclylalkyl group; Y is an electronegative leaving group; R^2 is CO_2H, CH_2CO_2H, or esters, amides or isosteres thereof; Ar is an optionally substituted aryl group; and R^3 is hydrogen, an optionally substituted C_{1-6} alkyl, F_2, CN, aryl or R^3 is attached to Ar to form an unsaturated or partially saturated five or six membered fused ring having 0-2 heteroatoms. The compounds are useful for treating caspase-mediated diseases in mammals.

CASPASE INHIBITORS AND USES THEREOF

This application claims the benefit of US provisional patent application serial number 60/206,362 filed May 23, 2000 and US provisional patent application serial number 60/217,006 filed July 10, 2000.

Field of the Invention

This invention is in the field of medicinal chemistry and relates to novel compounds, and pharmaceutical compositions thereof, that inhibit caspases that mediate cell apoptosis and inflammation. The invention also relates to methods of using the compounds and pharmaceutical compositions of this invention to treat diseases where caspase activity is implicated.

Background of the Invention

Apoptosis, or programmed cell death, is a principal mechanism by which organisms eliminate unwanted cells. The deregulation of apoptosis, either excessive apoptosis or the failure to undergo it, has been implicated in a number of diseases such as cancer, acute inflammatory and autoimmune disorders, ischemic diseases and certain neurodegenerative disorders (see generally *Science*, 1998, **281**, 1283-1312; Ellis et al., *Ann. Rev. Cell. Biol.*, 1991, **7**, 663).

Caspases are a family of cysteine protease enzymes that are key mediators in the signaling pathways for apoptosis and cell disassembly (Thornberry, *Chem.*

Biol., 1998, **5**, R97-R103). These signaling pathways vary depending on cell type and stimulus, but all apoptosis pathways appear to converge at a common effector pathway leading to proteolysis of key proteins. Caspases are involved in both the effector phase of the signaling pathway and further upstream at its initiation. The upstream caspases involved in initiation events become activated and in turn activate other caspases that are involved in the later phases of apoptosis.

Caspase-1, the first identified caspase, is also known as interleukin converting enzyme or "ICE." Caspase-1 converts precursor interleukin-1β ("pIL-1β") to the pro-inflammatory active form by specific cleavage of pIL-1β between Asp-116 and Ala-117. Besides caspase-1 there are also eleven other known human caspases, all of which cleave specifically at aspartyl residues. They are also observed to have stringent requirements for at least four amino acid residues on the N-terminal side of the cleavage site.

The caspases have been classified into three groups depending on the amino acid sequence that is preferred or primarily recognized. The group of caspases, which includes caspases 1, 4, and 5, has been shown to prefer hydrophobic aromatic amino acids at position 4 on the N-terminal side of the cleavage site. Another group which includes caspases 2, 3 and 7, recognize aspartyl residues at both positions 1 and 4 on the N-terminal side of the cleavage site, and preferably a sequence of Asp-Glu-X-Asp. A third group, which includes caspases 6, 8, 9 and 10, tolerate many amino acids in the primary recognition sequence, but seem to prefer residues with

branched, aliphatic side chains such as valine and leucine at position 4.

The caspases have also been grouped according to their perceived function. The first subfamily consists of caspases-1 (ICE), 4, and 5. These caspases have been shown to be involved in pro-inflammatory cytokine processing and therefore play an important role in inflammation. Caspase-1, the most studied enzyme of this class, activates the IL-1β precursor by proteolytic cleavage. This enzyme therefore plays a key role in the inflammatory response. Caspase-1 is also involved in the processing of interferon gamma inducing factor (IGIF or IL-18) which stimulates the production of interferon gamma, a key immunoregulator that modulates antigen presentation, T-cell activation and cell adhesion.

The remaining caspases make up the second and third subfamilies. These enzymes are of central importance in the intracellular signaling pathways leading to apoptosis. One subfamily consists of the enzymes involved in initiating events in the apoptotic pathway, including transduction of signals from the plasma membrane. Members of this subfamily include caspases-2, 8, 9 and 10. The other subfamily, consisting of the effector capsases 3, 6 and 7, are involved in the final downstream cleavage events that result in the systematic breakdown and death of the cell by apoptosis. Caspases involved in the upstream signal transduction activate the downstream caspases, which then disable DNA repair mechanisms, fragment DNA, dismantle the cell cytoskeleton and finally fragment the cell.

Knowledge of the four amino acid sequences primarily recognized by the caspases has been used to

design caspase inhibitors. Reversible tetrapeptide inhibitors have been prepared having the structure CH_3CO-[P4]-[P3]-[P2]-CH(R)CH_2CO_2H where P2 to P4 represent an optimal amino acid recognition sequence and R is an aldehyde, nitrile or ketone capable of binding to the caspase cysteine sulfhydryl. Rano and Thornberry, *Chem. Biol.* **4**, 149-155 (1997); Mjalli, et al., *Bioorg. Med. Chem. Lett.* **3**, 2689-2692 (1993); Nicholson et al., *Nature* **376**, 37-43 (1995). Irreversible inhibitors based on the analogous tetrapeptide recognition sequence have been prepared where R is an acyloxymethylketone $-COCH_2OCOR'$. R' is exemplified by an optionally substituted phenyl such as 2,6-dichlorobenzoyloxy and where R is $COCH_2X$ where X is a leaving group such as F or Cl. Thornberry et al., *Biochemistry* **33**, 3934 (1994); Dolle et al., *J Med. Chem.* 37, 563-564 (1994).

The utility of caspase inhibitors to treat a variety of mammalian disease states associated with an increase in cellular apoptosis has been demonstrated using peptidic caspase inhibitors. For example, in rodent models, caspase inhibitors have been shown to reduce infarct size and inhibit cardiomyocyte apoptosis after myocardial infarction, to reduce lesion volume and neurological deficit resulting from stroke, to reduce post-traumatic apoptosis and neurological deficit in traumatic brain injury, to be effective in treating fulminant liver destruction, and to improve survival after endotoxic shock. Yaoita et al., *Circulation*, **97**, 276 (1998); Endres et al., *J Cerebral Blood Flow and Metabolism*, **18**, 238, (1998); Cheng et al., J. Clin. Invest., **101**, 1992 (1998); Yakovlev et al., *J Neuroscience*, **17**, 7415 (1997); Rodriquez et al., *J. Exp.*

Med., **184**, 2067 (1996); Grobmyer et al., *Mol. Med.*, **5**, 585 (1999).

In general, the peptidic inhibitors described above are very potent against some of the caspase enzymes. However, this potency has not always been reflected in cellular models of apoptosis. In addition peptide inhibitors are typically characterized by undesirable pharmacological properties such as poor oral absorption, poor stability and rapid metabolism. Plattner and Norbeck, in *Drug Discovery Technologies*, Clark and Moos, Eds. (Ellis Horwood, Chichester, England, 1990).

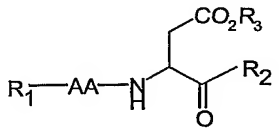
The unsuitable pharmacological properties of the tetra- and tri-peptidic caspase inhibitors has brought about the development of natural and non-natural amino acid di-peptidic inhibitors of caspases.

WO 91/15577 and WO 93/05071 disclose peptide ICE inhibitors of the formula:

$$Z\text{-}Q_2\text{-}Asp\text{-}Q_1$$

wherein Z is an N-terminal protecting group; Q_2 is 0 to 4 amino acids; and Q_1 is an electronegative leaving group. However, WO 91/15577 only reports these compounds to be active against caspase-1 and does not report activity against other caspases.

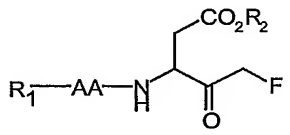
WO 99/18781 discloses dipeptide caspase inhibitors of the formula:



wherein R_1 is an N-terminal protecting group; AA is a residue of a natural α-amino acid, or β-amino acid; R_2 is

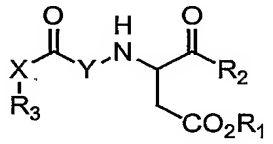
H or CH_2R_4 where R_4 is an electronegative leaving group; and R_3 is alkyl or H.

WO 99/47154 discloses dipeptide caspase inhibitors of the formula:



wherein R_1 is an N-terminal protecting group; AA is a residue of a non-natural α-amino acid, or β-amino acid; and R_2 is optionally substituted alkyl or H.

WO 00/61542 discloses dipeptide apoptosis inhibitors having the formula:



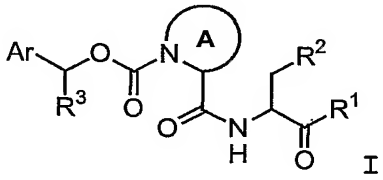
where R_1 is an optionally substituted alkyl or hydrogen group; R_2 is hydrogen or optionally substituted alkyl; Y is a residue of a natural or non-natural amino acid and R_3 is an alkyl, saturated carbocyclic, partially saturated carbocyclic, aryl, saturated heterocyclic, partially saturated heterocyclic or heteroaryl group, wherein said group is optionally substituted; X is O, S, NR_4, or $(CR_4R_5)_n$ where R_4 and R_5 are, at each occurrence, independently selected from the group consisting of hydrogen, alkyl and cycloalkyl, and n is 0, 1, 2, or 3; or X is NR_4, and R_3 and R_4 are taken together with the nitrogen atom to which they are attached to form a saturated heterocyclic, partially saturated heterocyclic or heteroaryl group, wherein said group is optionally substituted or X is CR_4R_5, and R_3 and R_4 are taken together

with the carbon atom to which they are attached to form a saturated carbocyclic, partially saturated carbocyclic, aryl, saturated heterocyclic, partially saturated heterocyclic or oxygen-containing heteroaryl group, wherein said group is optionally substituted; and provided that when X is O, then R_3 is not unsubstituted benzyl or t-butyl; and when X is CH_2, then R_3 is not H.

While a number of caspase inhibitors have been reported, it is not clear whether they possess the appropriate pharmacological properties to be therapeutically useful. Therefore, there is a continued need for small molecule caspase inhibitors that are potent, stable, and penetrate membranes to provide effective inhibition of apoptosis *in vivo*. Such compounds would be extremely useful in treating the aforementioned diseases where caspase enzymes play a role.

Summary of the Invention

It has now been found that compounds of this invention and pharmaceutical compositions thereof are particularly effective as inhibitors of caspases and cellular apoptosis. These compounds have the general formula **I**:



I

wherein:

Ring A is an optionally substituted piperidine, tetrahydroquinoline or tetrahydroisoquinoline ring;

R^1 is hydrogen, CHN_2, R, or $-CH_2Y$;

R is an optionally substituted group selected from an aliphatic group, an aryl group, an aralkyl group, a heterocyclic group, or an heterocyclylalkyl group;

Y is an electronegative leaving group;

R^2 is CO_2H, CH_2CO_2H, or esters, amides or isosteres thereof;

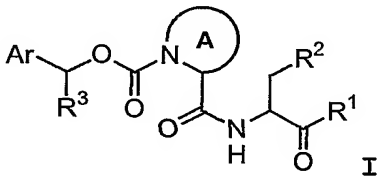
Ar is an optionally substituted aryl group; and

R^3 is hydrogen, an optionally substituted C_{1-6} alkyl, F_2, CN, aryl or R^3 is attached to Ar to form an unsaturated or partially saturated five or six membered fused ring having 0-2 heteroatoms.

The compounds of this invention have potent inhibition properties across a range of caspase targets with good efficacy in cellular models of apoptosis. In addition, these compounds are expected to have improved cell penetration and pharmacokinetic properties and, as a consequence of their potency, have improved efficacy against diseases where caspases are implicated.

Detailed Description of the Invention

This invention provides novel compounds, and pharmaceutically acceptable derivatives thereof, that are particularly effective as caspase inhibitors. The invention also provides methods for using the compounds to treat caspase-mediated disease states in mammals. The compounds have the general formula I:



I

wherein:

Ring A is an optionally substituted piperidine, tetrahydroquinoline or tetrahydroisoquinoline ring;

R^1 is hydrogen, CHN_2, R, or $-CH_2Y$;

R is an optionally substituted group selected from an aliphatic group, an aryl group, an aralkyl group, a heterocyclic group, or an heterocyclylalkyl group;

Y is an electronegative leaving group;

R^2 is CO_2H, CH_2CO_2H, or esters, amides or isosteres thereof;

Ar is an optionally substituted aryl group; and

R^3 is hydrogen, an optionally substituted C_{1-6} alkyl, F_2, CN, aryl or R^3 is attached to Ar to form an unsaturated or partially saturated five or six membered fused ring having 0-2 heteroatoms.

As used herein, the following definitions shall apply unless otherwise indicated. The term "aliphatic" as used herein means straight chained or branched C_1-C_{12} hydrocarbons which are completely saturated or which contain one or more units of unsaturation. Aliphatic groups include substituted or unsubstituted linear,

branched or cyclic alkyl, alkenyl, or alkynyl groups and hybrids thereof such as (cycloalkyl)alkyl, (cycloalkenyl)alkyl or (cycloalkyl)alkenyl. The term "aliphatic" includes "carbocyclic" groups. The term "alkyl" used alone or as part of a larger moiety refers to both straight and branched chains containing one to twelve carbon atoms. When the term alkyl is used as part of a larger moiety, as in aralkyl or heteroaralkyl, the alkyl portion will preferably contain one to six carbons.

The term "halogen" means F, Cl, Br, or I. The term "heteroatom" means nitrogen, oxygen or sulfur.

The term "aryl" refers to monocyclic or polycyclic aromatic groups, and monocyclic or polycyclic heteroaromatic groups containing one or more heteroatoms, having five to fourteen atoms. Such groups include, but are not restricted to phenyl, naphthyl, anthryl, furanyl, thienyl, pyrrolyl, oxazolyl, thiazolyl, imidazolyl, pyrazolyl, isoxazolyl, isothiazolyl, oxadiazolyl, triazolyl, thiadiazolyl, pyridinyl, pyridazinyl, pyrimidinyl, pyrazinyl, triazinyl, indolizinyl, indolyl, isoindolyl, indolinyl, benzofuranyl, benzothiophenyl, indazolyl, benzimidazolyl, benzthiazolyl, purinyl, quinolizinyl, quinolinyl, isoquinolinyl, cinnolinyl, phthalazinyl, quinazolinyl, quinoxalinyl, 1,8-naphthyridinyl, pteridinyl, carbazolyl, acridinyl, phenazinyl, phenothiazinyl, phenoxazinyl, tetrahydrofuranyl, phthalimidinyl, tetrazolyl, and chromanyl.

The term "heterocyclic group" refers to saturated and unsaturated monocyclic or polycyclic ring systems containing one or more heteroatoms and a ring size of three to eight. Such groups include, but are not

limited to aziranyl, oxiranyl, azetidinyl, tetrahydrofuranyl, pyrrolinyl, pyrrolidinyl, dioxolanyl, imidazolinyl, imidazolidinyl, pyrazolinyl, pyrazolidinyl, pyranyl, piperidinyl, dioxanyl, morpholinyl, dithianyl, thiomorpholinyl, piperazinyl, trithianyl, quinuclidinyl, oxepanyl, and thiepanyl.

The term "carbocyclic group" refers to saturated monocyclic or polycyclic carbon ring systems which may be fused to aryl or heterocyclic groups. Examples could include cyclohexyl, cyclopentyl, cyclobutyl, cyclopropyl, indanyl, tetrahydronaphthyl and the like.

An aliphatic, aryl, or heterocyclyl group may contain one or more substituents. Examples of suitable substituents include a halogen, -R, -OR, -OH, -SH, -SR, protected OH (such as acyloxy), phenyl (Ph), substituted Ph, -OPh, substituted -OPh, $-NO_2$, -CN, $-NH_2$, -NHR, $-N(R)_2$, -NHCOR, -NHCONHR, $-NHCON(R)_2$, -NRCOR, $-NHCO_2R$, $-CO_2R$, $-CO_2H$, -COR, -CONHR, $-CON(R)_2$, $-S(O)_2R$, $-SONH_2$, -S(O)R, $-SO_2NHR$, $-NHS(O)_2R$, =O, =S, =NNHR, $=NNR_2$, =N-OR, =NNHCOR, $=NNHCO_2R$, $=NNHSO_2R$, or =NR where R is an aliphatic group or a substituted aliphatic group.

A substitutable nitrogen on a heterocyclic ring may be optionally substituted. Suitable substituents on the nitrogen include R, COR, $S(O)_2R$, and CO_2R, where R is an aliphatic group or a substituted aliphatic group.

Nitrogen and sulfur may be in their oxidized form, and nitrogen may be in a quaternized form.

The term "electronegative leaving group" has the definition known to those skilled in the art (see March, Advanced Organic Chemistry, 4th Edition, John Wiley & Sons, 1992). Examples of electronegative leaving

groups include halogens such as F, Cl, Br, I, aryl- and alkylsulfonyloxy groups, trifluoromethanesulfonyloxy, OR, SR, -OC=O(R), -OPO(R^4)(R^5), where R is an aliphatic group, an aryl group, an aralkyl group, a carbocyclic group, an alkyl carbocyclic group, a heterocyclic group, or an alkyl heterocyclic group; and R^4 and R^5 are independently selected from R or OR.

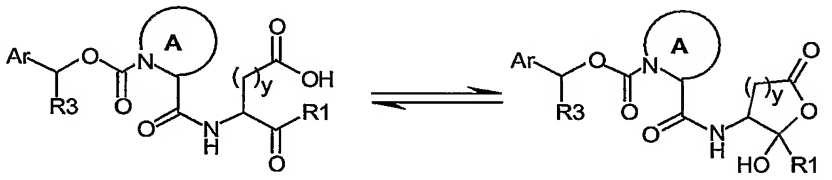
When the R^2 group is in the form of an ester or amide, the present compounds undergo metabolic cleavage to the corresponding carboxylic acids, which are the active caspase inhibitors. Because they undergo metabolic cleavage, the precise nature of the ester or amide group is not critical to the working of this invention. The structure of the R^2 group may range from the relatively simple diethyl amide to a steroidal ester. Examples of esters of R^2 carboxylic acids include, but are not limited to, C_{1-12} aliphatic, such as C_{1-6} alkyl or C_{3-10} cycloalkyl, aryl, such as phenyl, aralkyl, such as benzyl or phenethyl, heterocyclyl or heterocyclylalkyl. Examples of suitable R^2 heterocyclyl rings include, but are not limited to, 5-6 membered heterocyclic rings having one or two heteroatoms such as piperidinyl, piperazinyl, or morpholinyl.

Amides of R^2 carboxylic acids may be primary, secondary or tertiary. Suitable substituents on the amide nitrogen include, but are not limited to, one or more groups independently selected from the aliphatic, aryl, aralkyl, heterocyclyl or heterocyclylalkyl groups described above for the R^2 ester alcohol. Likewise, other prodrugs are included within the scope of this invention. See Bradley D. Anderson, "Prodrugs for Improved CNS

Delivery" in Advanced Drug Delivery Reviews (1996), **19**, 171-202.

Isosteres or bioisosteres of carboxylic acids and esters result from the exchange of an atom or group of atoms to create a new compound with similar biological properties to the parent carboxylic acid or ester. The bioisosteric replacement may be physicochemically or topologically based. An example of an isosteric replacement for a carboxylic acid is $CONHSO_2$(alkyl) such as $CONHSO_2Me$.

Compounds of this invention where R^2 is CO_2H or CH_2CO_2H, γ-ketoacids or δ-ketoacids respectively, may exist in solution as either the open form **(a)** or the cyclized hemiketal form **(b)** (y=1 for γ-ketoacids, y=2 for δ-ketoacids). The representation herein of either isomeric form is meant to include the other.



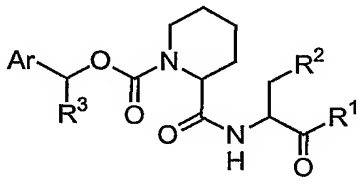
a **b**

Likewise it will be apparent to one skilled in the art that certain compounds of this invention may exist in tautomeric forms or hydrated forms, all such forms of the compounds being within the scope of the invention. Unless otherwise stated, structures depicted herein are also meant to include all stereochemical forms of the structure; i.e., the R and S configurations for each asymmetric center. Therefore, single stereochemical

isomers as well as enantiomeric and diastereomeric mixtures of the present compounds are within the scope of the invention. Unless otherwise stated, structures depicted herein are also meant to include compounds that differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the present structures except for the replacement of a hydrogen by a deuterium or tritium, or the replacement of a carbon by a ^{13}C- or ^{14}C-enriched carbon are within the scope of this invention.

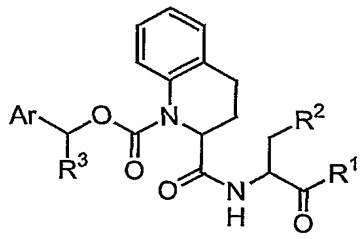
A number of dipeptidic ICE/caspase inhibitors that were generically and specifically described in WO 91/15557, WO 99/47154 and WO 00/61542 were tested for activity against caspases in the enzymatic and cell-based assays described below. The new compounds of formula **I** were found to have unexpectedly better activity relative to the previously described inhibitors.

Compounds of this invention wherein Ring A is an optionally substituted piperidine ring are represented by formula **Ia** below:



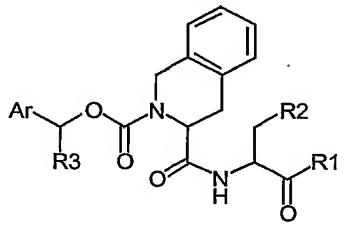
Ia

Compounds of this invention wherein Ring A is an optionally substituted tetrahydroquinoline ring are represented by formula **Ib** below:



Ib

Compounds of this invention wherein Ring A is an optionally substituted tetrahydroisoquinoline ring are represented by formula **Ic** below:



Ic

Ring A may be substituted or unsubstituted. Examples of suitable Ring A substituents include one or more groups selected from halogen, -R, -OR, -OH, -SR, protected OH (such as acyloxy), phenyl (Ph), substituted Ph, -OPh, substituted -OPh, $-NO_2$, -CN, $-NH_2$, -NHR, $-N(R)_2$, -NHCOR, -NHCONHR, $-NHCON(R)_2$, -NRCOR, $-NHCO_2R$, $-CO_2R$, $-CO_2H$, -COR, -CONHR, $-CON(R)_2$, $-S(O)_2R$, $-SONH_2$, $-S(O)R$, $-SO_2NHR$, $-NHS(O)_2R$, =O, =S, =NNHR, $=NNR_2$, =N-OR, =NNHCOR, $=NNHCO_2R$, $=NNHSO_2R$, or =NR where R is an aliphatic group or a substituted aliphatic group.

Preferred compounds of this invention are compounds of formula **I** that have one or more of the

following features and more preferably all of the following features:

(a) R^1 is a halomethyl group, more preferably CH_2F;

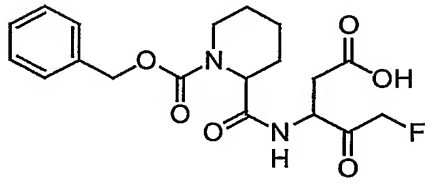
(b) R^2 is CO_2H or esters, amides or isosteres thereof;

(c) R^3 is hydrogen, C_{1-6} alkyl, or C_{1-6} haloalkyl, more preferably CF_3 or C_2F_5; and

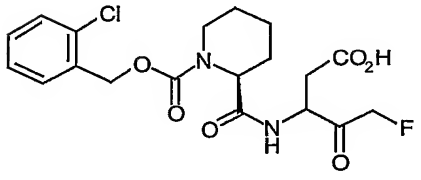
(d) Ar is an optionally substituted aryl, more preferably an optionally substituted phenyl.

Examples of specific compounds are shown below in Table 1.

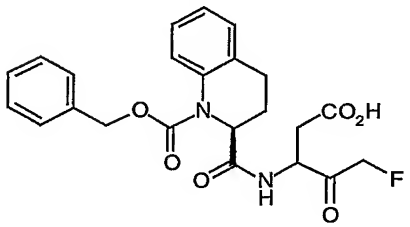
Table 1



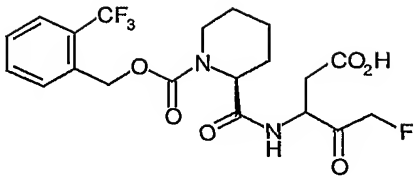
Example 1



Example 2



Example 3



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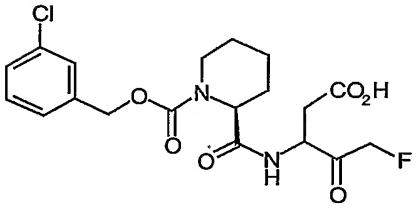

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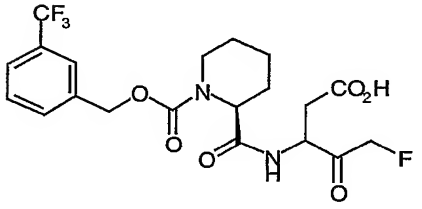

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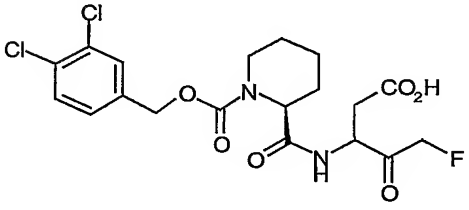

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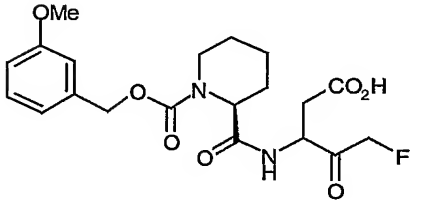

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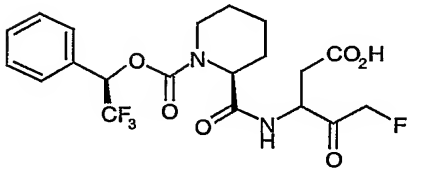

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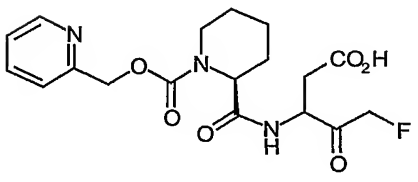

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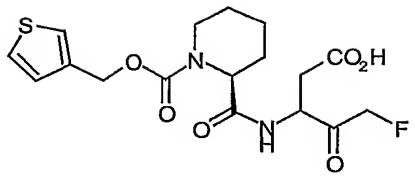

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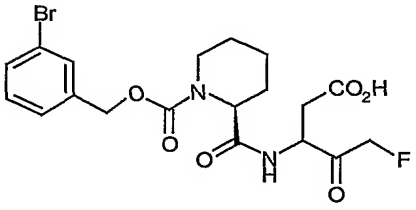

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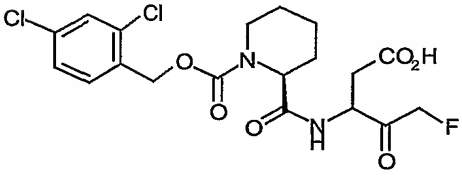

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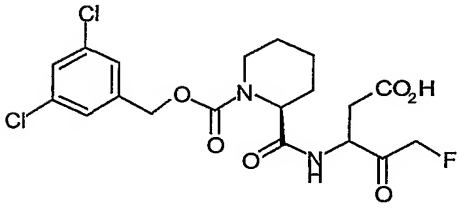

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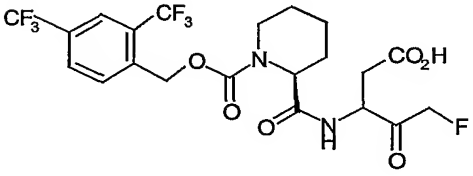

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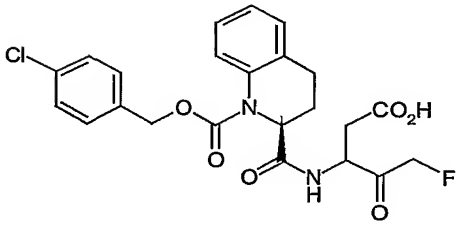

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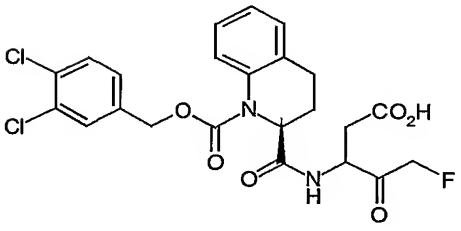

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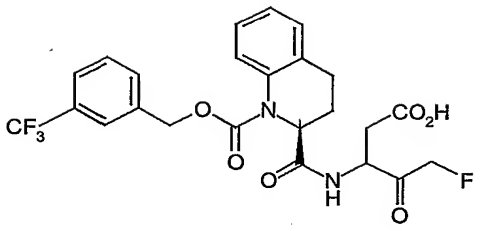

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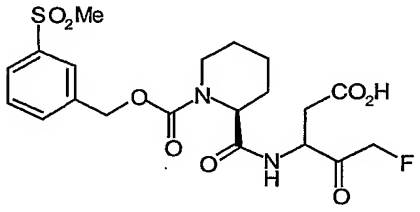

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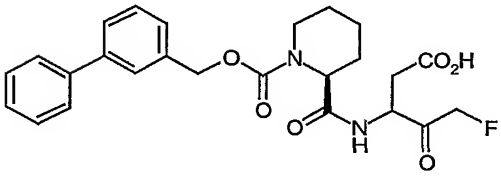

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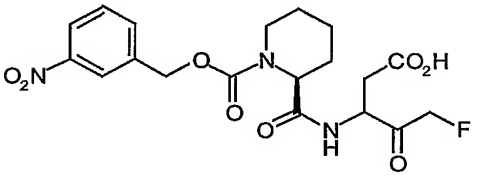

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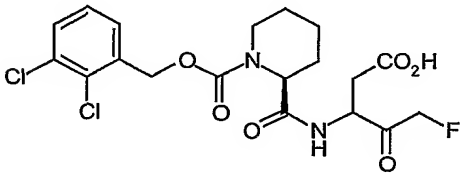

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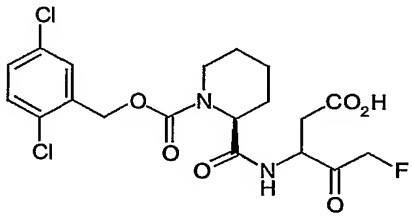

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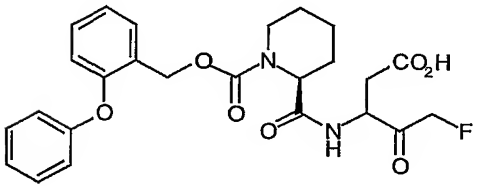

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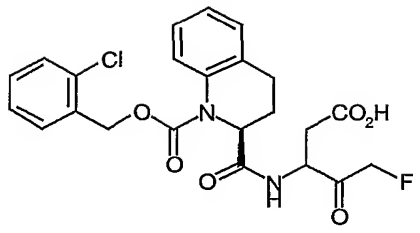

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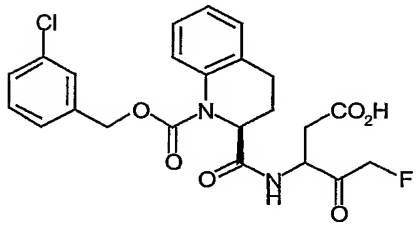

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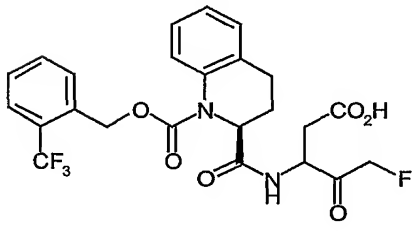

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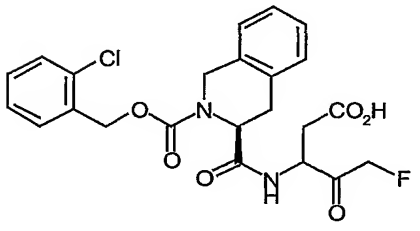

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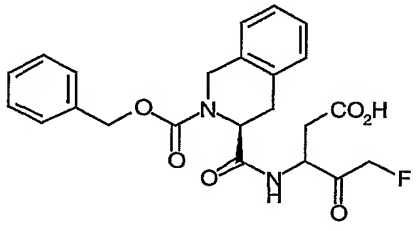

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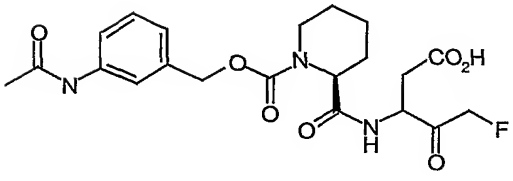

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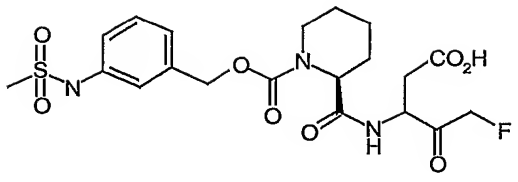

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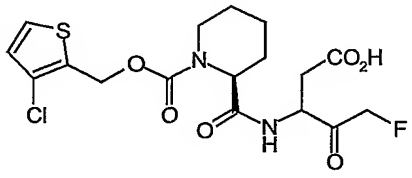

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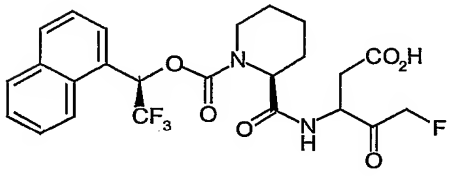

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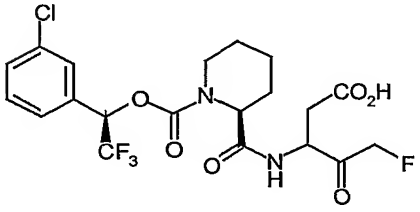

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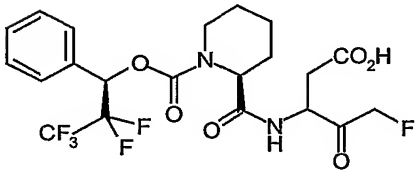

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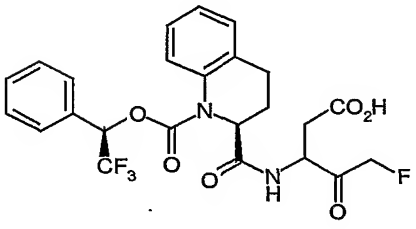

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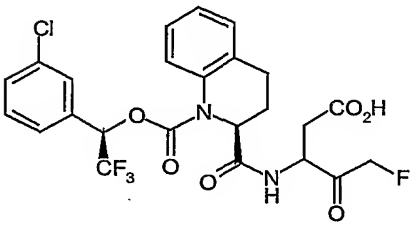

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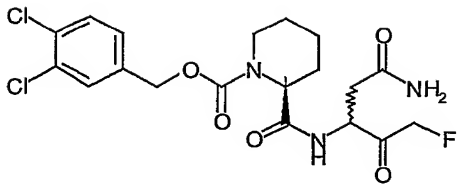

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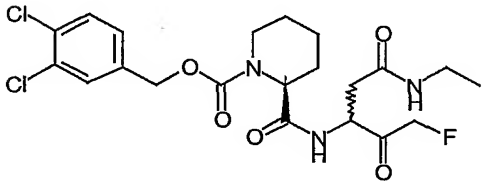

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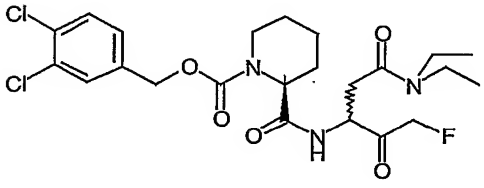

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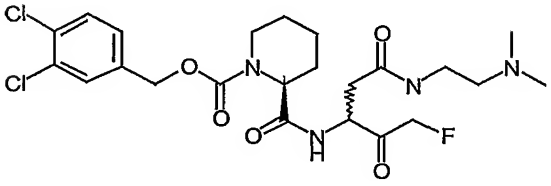

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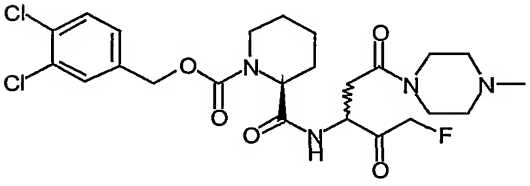

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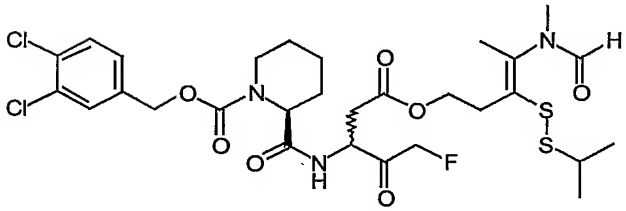

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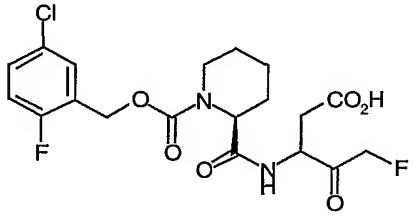
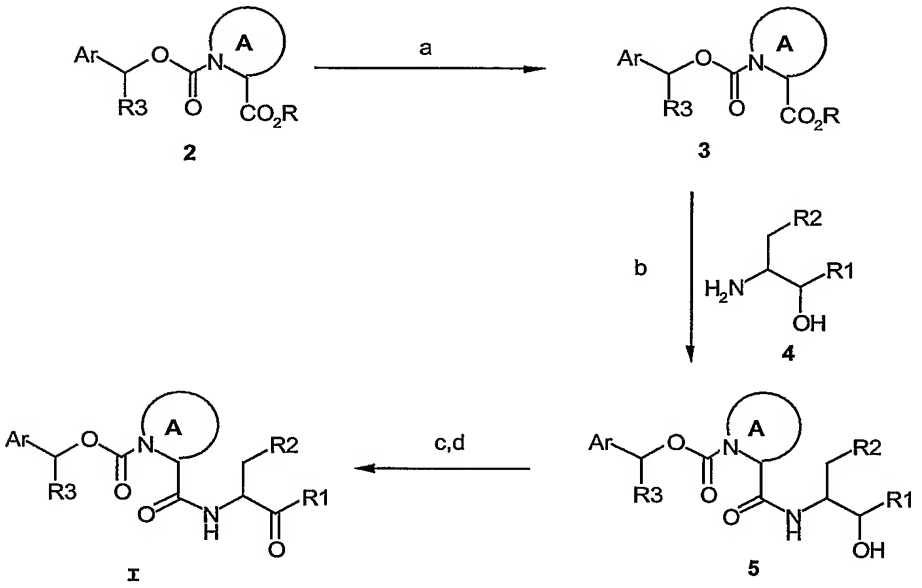


Example 44

The compounds of this invention may be prepared in general by methods known to those skilled in the art for analogous compounds, as illustrated by the general Scheme I below and by the preparative examples that follow.

Scheme I



Details: (a) TFA or KOH/MeOH; (b) EDC/DMAP/HOBt; (c) Dess-Martin periodinane; (d) TFA/DCM

In Scheme I above, the starting carbamate ester **2** (R is any suitable organic radical) is readily obtained by a carbamoylation reaction between the corresponding alcohol, $ArCH(OH)-R^3$, and the corresponding ester of piperidine-2-carboxylic acid, 1,2,3,4-tetrahydroquinoline-2-carboxylic acid or 1,2,3,4-tetrahydroisoquinoline-2-carboxylic acid. Such carbamate-forming reactions generally use phosgene or an equivalent thereof and are known to those skilled in the

art (i.e. formation of an intermediate carbamoyl chloride from the amine, then reaction with the alcohol; or formation of an intermediate chloroformate from the alcohol, then reaction with the amine; or formation of an intermediate chloroformate from the alcohol, then reaction with the amine). Carbamate ester **2** is hydrolyzed using base or, when the ester is a t-butyl group, using trifluoroacetic acid. The acid **3** is then coupled with the amino alcohol **4**. Depending on the nature of R^1 and R^2 an amino ketone may be used, in place of the amino alcohol, which avoids the need for a subsequent oxidation step. In the case of fluoromethyl ketones where R^1 is CH_2F, the amino alcohol **4** may be obtained according to the method of Revesz et al., *Tetrahedron Lett.*, **1994**, *35*, 9693. Finally the hydroxyl in compound **5** is oxidized and the compound treated appropriately according to the nature of R^2. For example, if the product **I** requires R^2 to be a carboxylic acid, then R^2 in **4** is preferably an ester and the final step in the scheme is acid or base-catalyzed deprotection .

The compounds of this invention are designed to inhibit caspases. Therefore, the compounds of this invention may be assayed for their ability to inhibit caspase activity, apoptosis, and the release of IL-1β directly. Assays for each of the activities are known in the art and are described below in detail in the Testing section.

According to another embodiment, the invention provides a composition comprising a compound of this invention or a pharmaceutically acceptable salt thereof, as described above, and a pharmaceutically acceptable carrier.

If pharmaceutically acceptable salts of the compounds of this invention are utilized in these compositions, those salts are preferably derived from inorganic or organic acids and bases. Included among such acid salts are the following: acetate, adipate, alginate, aspartate, benzoate, benzene sulfonate, bisulfate, butyrate, citrate, camphorate, camphor sulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, pamoate, pectinate, persulfate, 3-phenyl-propionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate and undecanoate. Base salts include ammonium salts, alkali metal salts, such as sodium and potassium salts, alkaline earth metal salts, such as calcium and magnesium salts, salts with organic bases, such as dicyclohexylamine salts, N-methyl-D-glucamine, and salts with amino acids such as arginine, lysine, and so forth.

Also, the basic nitrogen-containing groups can be quaternized with such agents as lower alkyl halides, such as methyl, ethyl, propyl, and butyl chloride, bromides and iodides; dialkyl sulfates, such as dimethyl, diethyl, dibutyl and diamyl sulfates, long chain halides such as decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides, aralkyl halides, such as benzyl and phenethyl bromides and others. Water or oil-soluble or dispersible products are thereby obtained.

The compounds utilized in the compositions and methods of this invention may also be modified by appending appropriate functionalities to enhance selective biological properties. Such modifications are known in the art and include those which increase biological penetration into a given biological system (e.g., blood, lymphatic system, central nervous system), increase oral availability, increase solubility to allow administration by injection, alter metabolism and alter rate of excretion.

Pharmaceutically acceptable carriers that may be used in these compositions include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat.

According to a preferred embodiment, the compositions of this invention are formulated for pharmaceutical administration to a mammal, preferably a human being.

Such pharmaceutical compositions of the present invention may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally,

vaginally or via an implanted reservoir. The term "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional and intracranial injection or infusion techniques. Preferably, the compositions are administered orally or intravenously.

Sterile injectable forms of the compositions of this invention may be aqueous or oleaginous suspension. These suspensions may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or di-glycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, such as carboxymethyl cellulose or similar dispersing agents which are commonly used in the formulation of pharmaceutically acceptable dosage forms including emulsions and suspensions. Other commonly used

surfactants, such as Tweens, Spans and other emulsifying agents or bioavailability enhancers which are commonly used in the manufacture of pharmaceutically acceptable solid, liquid, or other dosage forms may also be used for the purposes of formulation.

The pharmaceutical compositions of this invention may be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, aqueous suspensions or solutions. In the case of tablets for oral use, carriers that are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried cornstarch. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening, flavoring or coloring agents may also be added.

Alternatively, the pharmaceutical compositions of this invention may be administered in the form of suppositories for rectal administration. These can be prepared by mixing the agent with a suitable non-irritating excipient which is solid at room temperature but liquid at rectal temperature and therefore will melt in the rectum to release the drug. Such materials include cocoa butter, beeswax and polyethylene glycols.

The pharmaceutical compositions of this invention may also be administered topically, especially when the target of treatment includes areas or organs readily accessible by topical application, including

diseases of the eye, the skin, or the lower intestinal tract. Suitable topical formulations are readily prepared for each of these areas or organs.

Topical application for the lower intestinal tract can be effected in a rectal suppository formulation (see above) or in a suitable enema formulation. Topically-transdermal patches may also be used.

For topical applications, the pharmaceutical compositions may be formulated in a suitable ointment containing the active component suspended or dissolved in one or more carriers. Carriers for topical administration of the compounds of this invention include, but are not limited to, mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax and water. Alternatively, the pharmaceutical compositions can be formulated in a suitable lotion or cream containing the active components suspended or dissolved in one or more pharmaceutically acceptable carriers. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

For ophthalmic use, the pharmaceutical compositions may be formulated as micronized suspensions in isotonic, pH adjusted sterile saline, or, preferably, as solutions in isotonic, pH adjusted sterile saline, either with our without a preservative such as benzylalkonium chloride. Alternatively, for ophthalmic uses, the pharmaceutical compositions may be formulated in an ointment such as petrolatum.

The pharmaceutical compositions of this invention may also be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other conventional solubilizing or dispersing agents.

The above-described compositions are particularly useful in therapeutic applications relating to an IL-1 mediated disease, an apoptosis mediated disease, an inflammatory disease, an autoimmune disease, a destructive bone disorder, a proliferative disorder, an infectious disease, a degenerative disease, a disease associated with cell death, an excess dietary alcohol intake disease, a viral mediated disease, uveitis, inflammatory peritonitis, osteoarthritis, pancreatitis, asthma, adult respiratory distress syndrome, glomerulonephritis, rheumatoid arthritis, systemic lupus erythematosus, scleroderma, chronic thyroiditis, Grave's disease, autoimmune gastritis, diabetes, autoimmune hemolytic anemia, autoimmune neutropenia, thrombocytopenia, chronic active hepatitis, myasthenia gravis, inflammatory bowel disease, Crohn's disease, psoriasis, atopic dermatitis, scarring, graft vs host disease, organ transplant rejection, osteoporosis, leukemias and related disorders, myelodysplastic syndrome, multiple myeloma-related bone disorder, acute myelogenous leukemia, chronic myelogenous leukemia, metastatic melanoma, Kaposi's sarcoma, multiple myeloma, haemorrhagic shock, sepsis, septic shock, burns,

Shigellosis, Alzheimer's disease, Parkinson's disease, Huntington's disease, Kennedy's disease, prion disease, cerebral ischemia, epilepsy, myocardial ischemia, acute and chronic heart disease, myocardial infarction, congestive heart failure, atherosclerosis, coronary artery bypass graft, spinal muscular atrophy, amyotrophic lateral sclerosis, multiple sclerosis, HIV-related encephalitis, aging, alopecia, neurological damage due to stroke, ulcerative colitis, traumatic brain injury, spinal cord injury, hepatitis-B, hepatitis-C, hepatitis-G, yellow fever, dengue fever, or Japanese encephalitis, various forms of liver disease including alcoholic hepatitis, renal disease, polyaptic kidney disease, H. pylori-associated gastric and duodenal ulcer disease, HIV infection, tuberculosis, and meningitis. The compounds and compositions are also useful in treating complications associated with coronary artery bypass grafts and as a component of immunotherapy for the treatment of various forms of cancer.

The amount of compound present in the above-described compositions should be sufficient to cause a detectable decrease in the severity of the disease or in caspase activity and/or cell apoptosis, as measured by any of the assays described in the examples.

The compounds of this invention are also useful in methods for preserving cells, such as may be needed for an organ transplant or for preserving blood products. Similar uses for caspase inhibitors have been reported (Schierle et al., Nature Medicine, 1999, **5**, 97). The method involves treating the cells or tissue to be preserved with a solution comprising the caspase inhibitor. The amount of caspase inhibitor needed will

depend on the effectiveness of the inhibitor for the given cell type and the length of time required to preserve the cells from apoptotic cell death.

According to another embodiment, the compositions of this invention may further comprise another therapeutic agent. Such agents include, but are not limited to, thrombolytic agents such as tissue plasminogen activator and streptokinase. When a second agent is used, the second agent may be administered either as a separate dosage form or as part of a single dosage form with the compounds or compositions of this invention.

It should also be understood that a specific dosage and treatment regimen for any particular patient will depend upon a variety of factors, including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, rate of excretion, drug combination, and the judgment of the treating physician and the severity of the particular disease being treated. The amount of active ingredients will also depend upon the particular compound and other therapeutic agent, if present, in the composition.

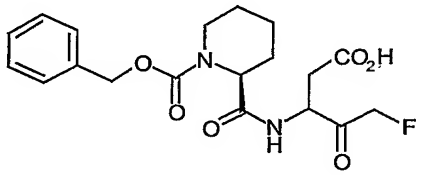
In a preferred embodiment, the invention provides a method of treating a mammal, having one of the aforementioned diseases, comprising the step of administering to said mammal a pharmaceutically acceptable composition described above. In this embodiment, if the patient is also administered another therapeutic agent or caspase inhibitor, it may be delivered together with the compound of this invention in a single dosage form, or, as a separate dosage form.

When administered as a separate dosage form, the other caspase inhibitor or agent may be administered prior to, at the same time as, or following administration of a pharmaceutically acceptable composition comprising a compound of this invention.

In order that this invention be more fully understood, the following preparative and testing examples are set forth. These examples are for the purpose of illustration only and are not to be construed as limiting the scope of the invention in any way.

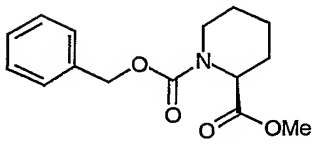
Synthetic Examples

[3S/R, (2S)]-3-(1-Benzyloxycarbonyl-2-piperidinecarboxamido)-5-fluoro-4-oxo-pentanoic acid (Example 1)



Method A:

(S)-Piperidine-1,2-dicarboxylic acid 1-benzyl ester 2-methyl ester

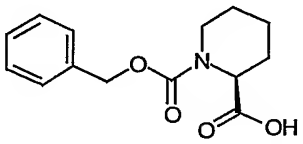


To a stirred suspension of (S)-piperidine-2-carboxylic acid methyl ester hydrochloride salt (5.0g, 27.8mmol) in dichloromethane (DCM) (35mL) at 0°C was added diisopropylethylamine (DIPEA) (10.1mL, 58.4mmol) followed

by N-(benzyloxycarbonyloxy)succinimide (7.63g, 30.6mmol). The reaction mixture was allowed to warm to room temperature and stirred overnight. The residue was diluted with DCM and washed with 1.0-M HCl. The organic layer was washed with brine, dried (Na_2SO_4), filtered and concentrated *in vacuo*. The residue was purified by flash chromatography (20% ethyl acetate in hexane) to afford the title compound as a pale yellow oil (7.72, 100%): ^1H NMR (400MHz, CD_3OD) δ 1.19-1.88 (5H, m), 2.15-2.28 (1H, m), 2.91-3.12 (1H, m), 3.70-3.73 (3H, 2s), 4.00-4.07 (1H, m), 4.82-4.87 (1H, m), 5.03-5.21 (2H, m), 7.24-7.39 (5H, m); ^{13}C NMR (100MHz, CD_3OD) δ 22.0, 22.1 (CH_2), 26.0, 26.1 (CH_2), 28.1 (CH_2), 43.4, 43.5 (CH_2), 53.1 (CH_3), 56.2, 56.5 (CH), 68.8, 68.9 (CH_2), 129.2 (CH), 129.5 (CH), 129.9 (CH), 138.4 (C), 167.0 (CO), 173.7 (CO).

Method B:

(S)-Piperidine-1,2-dicarboxylic acid 1-benzyl ester

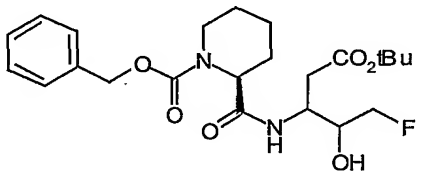


To a stirred solution of (S)-piperidine-1,2-dicarboxylic acid 1-benzyl ester 2-methyl ester (8.0g, 28.8mmol) in MeOH (75mL) and water (38mL) at 0°C was added powdered KOH (1.78g, 31.7mmol). The reaction mixture was allowed to stir for 16h at room temperature and then the MeOH was removed *in vacuo*. The residue was diluted with water and washed DCM. The aqueous layer was acidified with 1.0-M HCl and extracted with ethyl acetate three times. The combined organic extracts were dried (Na_2SO_4), filtered and concentrated *in vacuo* to afford the title compound as

pale yellow oil (7.6 g, 100%): ^{1}H NMR (400MHz, CD_3OD) δ 1.36-1.61 (2H, m), 1.70-1.85 (3H, m), 2.28-2.40 (1H, m), 3.05-3.26 (1H, m), 4.06-4.14 (1H, m), 4.89-5.26 (4H, m), 7.37-7.48 (5H, m); ^{13}C NMR (100MHz, CD_3OD) □ 20.5, 20.6 (CH_2), 24.6, 24.7 (CH_2), 26.6 (CH_2), 41.8, 41.9 (CH_2), 54.5, 54.7 (CH), 67.2, 67.3 (CH_2), 127.6 (CH), 127.9 (CH), 128.4 (CH), 136.9 (C), 173.4 (CO).

Method C:

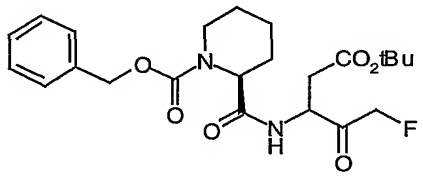
[3S/R, (2S)]-3-(1-Benzyloxycarbonyl-2-piperidinecarboxamido)-5-fluoro-4-hydroxy-pentanoic acid tert-butyl ester



A stirred mixture of (S)-piperidine-1,2-dicarboxylic acid 1-benzyl ester (606mg, 2.30mmol), 3-amino-5-fluoro-4-hydroxy-pentanoic acid *tert*-butyl ester (500mg, 2.42mmol), HOBT (344mg, 2.53mmol), DMAP (323mg, 2.65mmol) and anhydrous THF (17mL) was cooled to 0°C then EDC (485mg, 2.53mmol) was added. The mixture was allowed to warm to room temperature during 16h then concentrated under reduced pressure. The residue purified by flash chromatography (5% ethyl acetate in petroleum spirit - 50% ethyl acetate in petroleum spirit) to give the title compound as a colourless oil (871mg, 84%): ^{1}H NMR (400MHz, $CDCl_3$) δ 1.44 (9H, s), 1.50-3.09 (9H, m), 3.87-5.18 (9H, m), 6.72-7.04 (1H, m), 7.22-7.37 (5H, m); ^{19}F NMR (376MHz, $CDCl_3$) δ -229.1, -229.3, -230.0, -230.3, -230.5.

Method D:

[3S/R, (2S)]-3-(1-Benzyloxycarbonyl-2-piperidinecarboxamido)-5-fluoro-4-oxo-pentanoic acid tert-butyl ester

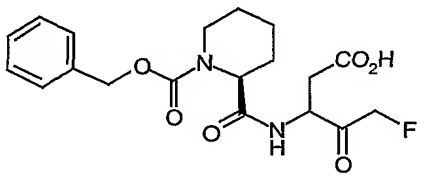


A stirred solution of [3S/R, (2S)]-3-(1-benzyloxycarbonyl-2-piperidinecarboxamido)-5-fluoro-4-hydroxy-pentanoic acid tert-butyl ester (871mg, 1.93mmol) in anhydrous dichloromethane (DCM) (40mL) was treated at 0°C with 1,1,1 triacetoxy-1,1-dihydro-1,2-beziodoxol-3(1*H*)-one (980mg, 2.31mmol). The mixture was stirred at room temperature for 16h, diluted with ethyl acetate and washed with a 1:1 mixture of aqueous $NaHSO_4$ and aqueous $Na_2S_2O_3$. The organic layer was collected, dried ($MgSO_4$) and concentrated. The residue was purified by flash chromatography (40% ethyl acetate in hexane) to give the title compound as a colourless oil (738mg, 85%): ^1H NMR (400 MHz, $CDCl_3$) δ 1.46 (9H, s), 1.39-1.74 (5H, m), 2.25-2.36 (1H, m), 2.70-3.06 (3H, m), 4.09-4.12 (1H, m), 4.80-5.19 (6H, m), 7.01-7.15 (1H, m), 7.28-7.37 (5H, m); ^{13}C NMR (100MHz, $CDCl_3$) δ 20.7 (CH_2), 25.1/26.0 (CH_2), 28.3 (CH_3), 36.6, 36.7 (CH_2), 42.5, 42.7 (CH_2), 52.8 (CH), 55.2 (CH), 68.1, ,68.2 (CH_2), 82.7 (C), 84.5, 84.6 (CH_2F), 128.3 (CH), 128.6 (CH), 129.0 (CH), 136.7 (C), 170.3, 170.4 (CO), 171.5 (CO), 202.0 (CO); ^{19}F NMR (376MHz, $CDCl_3$) δ -231.6, -231.9, -232.2.

Method E:

[3S/R, (2S)]-3-(1-Benzyloxycarbonyl-2-piperidinecarboxamido)-5-fluoro-4-oxo-pentanoic acid

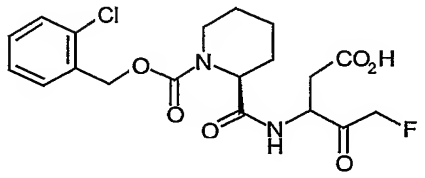
(Example 1)



Trifluoroacetic acid (TFA) (12mL) was added to a stirred ice cold solution of [3S/R,(2S)]-3-(1-benzyloxycarbonyl-2-piperidinecarboxamido)-5-fluoro-4-oxo-pentanoic acid tert-butyl ester (698mg, 1.55mmol) in anhydrous DCM (38mL). The mixture was stirred at 0°C for 0.5h then at room temperature for 0.5h. The mixture was concentrated under reduced pressure and then the residue was dissolved in dry DCM. This process was repeated several times in order to remove excess TFA. The gum was lyophilized twice from HPLC grade water / acetonitrile to afford the title compound as a white solid foam (481mg, 79%): IR (solid) 1736, 1665, 1517, 1436, 1255, 1174, 1041, 931cm^{-1}; ^1H NMR (400MHz, d_6-DMSO) δ 1.12-1.40 (2H, m), 1.45-1.68 (3H, m), 2.05 (1H, m), 2.61-2.63 (1H, m), 2.70-2.87 (1H, m), 2.98-3.21 (1H, m), 3.91 (1H, m), 4.28-4.75 (3H, m), 4.91-5.30 (3H, m), 7.25-7.42 (5H, m), 7.80-8.59 (1H, brm), 12.5 (1H, brs); ^{13}C NMR (100MHz, d_6-DMSO) δ (DMSO) 20.0 (CH_2), 24.6 (CH_2), 27.3 (CH_2), 34.7 (CH_2), 42.1 (CH_2), 52.1, 52.4 (CH), 54.5 (CH), 66.7 (CH_2), 84.2 (CH_2F), 127.8 (ArCH), 128.1 (ArCH), 128.7 (ArCH), 131.1 (ArC), 171.4 (CO), 172.0 (CO), 172.1 (CO), 202.8 (CO); ^{19}F NMR (376MHz,

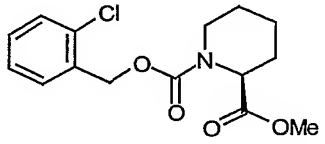
d_6-DMSO) δ -226.6, -226.8, -226.9, -232.4, -232.6, -232.7; MS (FAB +ve, HR) Calculated for $C_{19}H_{24}FN_2O_6$ (MH+) 395.1618, found 395.1625.

[3S/R, (2S)]-3-(1-(2-Chlorobenzyloxycarbonyl)-2-piperidinecarboxamido)-5-fluoro-4-oxo-pentanoic acid (Example 2)



Method F:

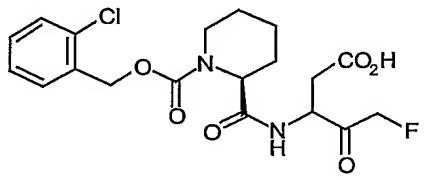
(S)-1-(2-Chlorobenzyloxycarbonyl)-piperidine-2-carboxylic acid methyl ester



To a vigorously stirred solution of (S)-pipecolic acid methyl ester hydrochloride (500mg, 2.79mmol) in dry DCM (10mL) cooled in an ice-bath, was added dropwise Et_3N (705mg, 6.96mmol) followed by 2-chlorobenzyl chloroformate (made from 2-chlorobenzyl alcohol using the method described in *J. Med. Chem.*, 1998, **41**, 1315-1343) (857mg, 4.18mmol). The resulting suspension was stirred at 0°C for a further 0.75h, diluted with ethyl acetate (30mL) and poured into 1.0-M HCl (30mL). The organic layer was separated and washed sequentially with 1.0-M HCl (20mL), aq.$NaHCO_3$ (20mL) and brine (20mL). The organic layer was then dried ($NaSO_4$),

filtered and concentrated under reduced pressure to give a colourless oil. The oil was purified by column chromatography (15% ethyl acetate in hexane) to give the title compound as a colorless viscous oil (824mg, 95%): ^{1}H NMR (400MHz, $CDCl_3$) 1.2-1.4 (1H, m), 1.4-1.6 (1H, m), 1.6-1.8 (3H, m), 2.2-2.3 (1H, m), 2.9-3.2 (1H, m), 3.7-3.8 (3H, m), 4.0-4.2 (1H, m), 4.8-5.0 (1H, m), 5.2-5.4 (2H, m), 7.2-7.3 (2H, m), 7.3-7.5 (2H, m)

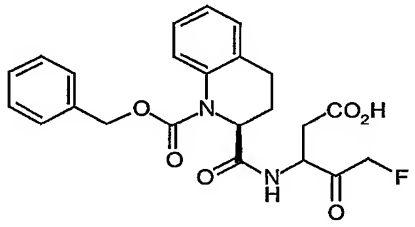
[3S/R, (2S)]-3-(1-(2-Chlorobenzyloxycarbonyl)-2-piperidinecarboxamido)-5-fluoro-4-oxo-pentanoic acid (Example 2)



This was prepared from (S)-1-(2-chlorobenzyloxycarbonyl)-piperidine-2-carboxylic acid methyl ester using procedures similar to those described above in Methods B-E (161mg, 70% last step): IR (solid) 1668, 1789 cm^{-1}; ^{1}H NMR (400MHz, d_6-DMSO) δ 1.1-1.8 (5H, m), 2.0-2.2 (1H, m), 2.4-2.9 (2H, m), 3.0-3.5 (1H, m), 3.8-4.0 (1H, m), 4.2-4.8 (3H, m), 5.0-5.4 (3H, m), 7.3-7.6 (4H, m), 7.8-8.7 (1H, m), 12.0-13.0 (1H, br s); ^{13}C NMR (100MHz, d_6-DMSO) δ 20.50, 20.77, 25.01, 25.14, 27.80, 28.18 (CH_2), 33.40, 35.20 (CH_2), 42.71 (CH_2), 47.89, 48.05, 52.65, 52.91, 53.35, 54.82, 55.05 (2 x CH), 64.66, 64.77 (CH_2), 81.94, 82.04, 83.70, 83.80, 85.60 (CH_2), 128.21, 130.11, 130.18, 130.40, 130.61 (ArCH), 133.08, 134.93, 134.95 (ArC),

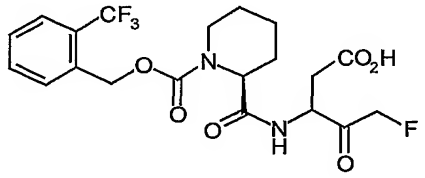
155.72, 156.40, 171.72, 172.20, 172.39, 172.58, 172.65, 173.83, 203.14, 203.29 (CO); ^{19}F NMR (376MHz, d_6-DMSO) δ -226.6, -226.8, -226.9, -230.2, -230.4, -232.4, -232.6, -232.6; Low Res MS ES+ 429.4, ES- 427.5.

[3S/R, (2S)]-3-(1-Benzyloxycarbonyl-1,2,3,4-tetrahydro-quinolinyl-2-carboxamido)-5-fluoro-4-oxo-pentanoic acid (Example 3)



This was prepared from (S)-1-benzyloxycarbonyl-1,2,3,4-tetrahydro-quinoline-2-carboxylic acid (US 4,461,896) using procedures similar to those described above in Methods C-E (142mg, 100%): IR (solid) 2981, 1684, 1653, 1522, 1492, 1394, 1323, 1207, 1053, 1018; ^{1}H NMR (400MHz, d_6-DMSO) δ 1.71 (1H, m), 2.29 (1H, m), 2.31-2.88 (4H, m), 4.00-5.30 (6H, m), 6.97 (1H, m), 7.12 (2H, m), 7.38 (5H, m), 7.69 (1H, m), 8.25+8.62+8.72 (1H, 3xm); ^{13}C NMR (100MHz, d_6-DMSO) δ 26.07, 26.20 (CH_2), 28.52, 28.76, 28.95 (CH_2), 35.13, 35.34 (CH_2), 52.54, 52.84, 53.21 (CH), 58.31, 58.35 (CH), 84.73 (FCH_2, J 177Hz), 124.34, 126.96, 128.27, 128.39, 128.45, 128.49, 128.55, 128.78, 128.83 (CH), 132.19, 12.41 (C), 137.04, 137.78 (C=O), 154.98 (C=O), 172.66, 172.73 (C=O), 203.00, 203.15, 203.29 (FCH_2C=O); ^{19}F NMR (376MHz, d_6-DMSO) δ -226.59 (t, J 45Hz), -226.91 (t, J 45Hz), -232.76 (t, J 45Hz).

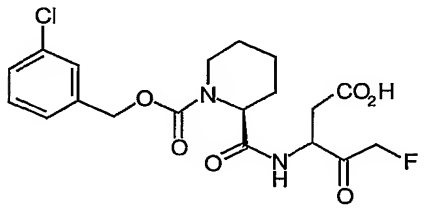
[3S/R, (2S)]-5-Fluoro-4-oxo-3-(1-(2-trifluoromethyl benzyloxycarbonyl)-2-piperidinecarboxamido)-pentanoic acid (Example 4)



This was prepared from 2-trifluoromethylbenzyl alcohol using procedures similar to those described above in Methods F, and B-E to give an off-white solid (150mg, 79% last step): IR (solid) 1672, 1737, 17867 cm^{-1}; ^1H NMR (400MHz, d_6-DMSO) δ 1.1-1.4 (2H, m), 1.5-1.7 (3H, m), 2.0-2.2 (1H, m), 2.5-2.6 (1H, m), 2.7-3.0 (1H, m), 3.0-3.4 (1H, m), 3.9-4.0 (1H, m), 4.3-4.8 (2.7H, m), 5.0-5.4 (3.3H, m), 7.5-7.9 (4H, m), 7.9-8.6 (1H, m); ^{13}C NMR (100MHz, d_6-DMSO) δ 19.98, 20.23, 24.50, 27.27, 27.62 (CH_2), 32.96, 34.65 (CH_2), 42.12 (CH_2), 47.45, 47.60, 52.15, 52.41, 52.89, 54.52 (2x CH), 63.11, 63.38 (CH_2), 81.48, 81.57, 83.32, 85.05(CH_2F), 120.53, 123.35, 125.97 (ArC), 126.35, 128.94, 130.13, 133.18 (ArCH), 135.07 (ArC), 171.56, 172.02, 172.07, 173.26, 174.14, 202.70 (CO); ^{19}F NMR (376MHz, d_6-DMSO) δ -59.3, -226.7, -226.7, -226.8, -226.9, -230.2, -230.5, -232.5, -232.6, -232.7, -232.7.

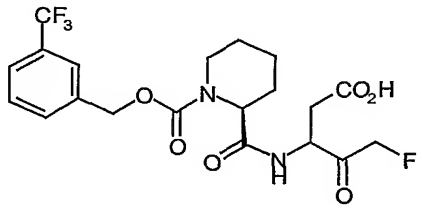
[3S/R, (2S)]-3-(1-(3-Chlorobenzyloxycarbonyl)-2-piperidinecarboxamido)-5-fluoro-4-oxo-pentanoic acid

(Example 5)



This was prepared from 3-chlorobenzyl alcohol using procedures similar to those described above in Methods F, and B-E to give an off-white solid (99mg, 54% last step): IR (solid) 1672, 1788 cm^{-1}; ^1H NMR (400MHz, d_6-DMSO) δ 1.1-1.4 (2H, m), 1.5-1.7 (3H, m), 2.0-2.2 (1H, m), 2.4-2.6 (1H, m), 2.6-2.9 (1H, m), 3.0-3.3 (1H, m), 3.8-4.0 (1H, m), 4.2-4.8 (2.7H, m), 5.0-5.3 (3.3H, m), 7.2-7.5 (4H, m), 7.5-8.7 (1H, m); ^{13}C NMR (100MHz, d_6-DMSO) δ 19.99, 20.24, 24.57, 27.09, 27.27, 27.63 (CH_2), 32.96, 34.72, 34.86 (CH_2), 42.14 (CH_2), 47.45, 47.61, 52.20, 52.40, 52.87, 54.53 (2x CH), 65.79 (CH_2), 81.51, 81.56, 83.28, 85.06 (CH_2F), 126.99, 127.50, 128.05, 130.65 (ArCH), 133.40, 139.72, 139.75 (ArC), 171.26, 171.80, 172.03, 172.08, 173.27, 174.14, 202.58, 202.72 (CO); ^{19}F NMR (376MHz, d_6-DMSO) δ -226.6, -226.8, -226.9, -230.2, -230.3, -232.4, -232.5, -232.6.

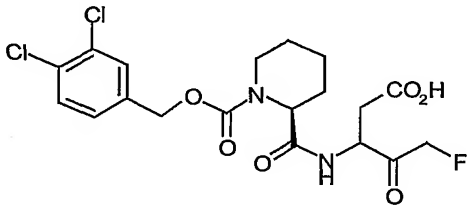
[3S/R, (2S)]-5-Fluoro-4-oxo-3-(1-(3-trifluoromethyl benzyloxycarbonyl)-2-piperidinecarboxamido)-pentanoic acid (Example 6)



This was prepared from 3-trifluoromethylbenzyl alcohol using procedures similar to those described above in Methods F, and B-E to give an off-white solid (142mg, 64% last step): IR (solid) 1670, 1788 cm^{-1}; ^1H NMR (400MHz, d_6-DMSO) δ 1.1-1.7 (5H, m), 2.0-2.2 (1H, m), 2.5-2.9 (2H, m), 3.0-3.3 (1H, m), 3.8-4.0 (1H, m), 4.2-4.8 (3H, m), 4.9-5.3 (3H, m), 7.5-7.8 (4H, m), 7.8-8.6 (1H, m); ^{13}C NMR (100MHz, d_6-DMSO) δ 20.02, 20.28, 24.63, 27.10, 27.28, 27.66 (CH_2), 32.86, 34.69 (CH_2), 42.19 (CH_2), 47.40, 47.57, 52.19, 52.40, 54.58 (2x CH), 65.29, 65.89 (CH_2Ar), 83.30, 85.02 (CH_2F), 124.20, 124.89, 129.91, 131.80 (4x ArCH), 138.71, 138.74 (2x ArC), 171.91, 172.09, 172.13, 173.33 (CO); ^{19}F NMR (376MHz, d_6-DMSO) δ -61.5, -226.7, -226.9, -226.9, -230.2, -230.4, -232.5, -232.6, -232.7; Low Res MS ES+ 461.3, ES- 463.2.

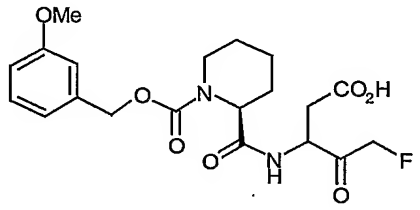
[3S/R, (2S)]-3-(1-(3,4-Dichlorobenzyloxycarbonyl)-2-piperidinecarboxamido)-5-fluoro-4-oxo-pentanoic acid

(Example 7)



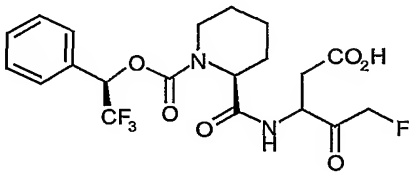
This was prepared from 3,4-dichlorobenzyl alcohol using procedures similar to those described above in Methods F, and B-E to give an off-white solid (167mg, 70% last step): IR (solid) 1671, 1785 cm^{-1}; ^1H NMR (400MHz, d_6-DMSO) δ 1.1-1.8 (5H, m), 2.0-2.2 (1H, m), 2.5-2.9 (2H, m), 3.0-3.3 (1H, m), 3.8-4.0 (1H, m), 4.2-4.8 (2.5H, m), 5.0-5.4 (3.5H, m), 7.2-7.4 (1H, m), 7.5-7.7 (2H, m), 7.7-8.6 (1H, m); ^{13}C NMR (100MHz, d_6-DMSO) δ 19.99, 24.45, 24.61, 27.26, 27.61 (3x CH_2), 32.88, 34.68 (CH_2), 42.71 (CH_2), 47.42, 47.57, 52.20, 52.40, 54.52 (2x CH), 65.12, 65.27 (CH_2Ar), 81.53, 83.30, 85.08 (CH_2F), 127.87, 128.03, 129.59, 129.76 (ArCH), 130.64 (ArC), 131.01 (ArCH), 131.37, 138.39, 138.42 (ArC), 171.88, 172.07, 172.12, 173.32, 202.78 (CO); ^{19}F NMR (376MHz, d_6-DMSO) δ -226.6, -226.8, -226.9, -230.2, -230.3, -232.4, -232.6, -232.6.

[3S/R, (2S)]-5-Fluoro-3-(1-(3-methoxybenzyloxycarbonyl)-2-piperidinecarboxamido)-4-oxo-pentanoic acid (Example 8)



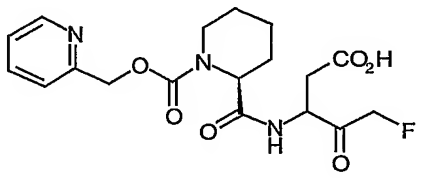
This was prepared from 3-methoxybenzyl alcohol using procedures similar to those described above in Methods F, and B-E to give an off-white solid (112mg, 58% last step): IR (solid) 1670, 1738, 1785 cm^{-1}; ^1H NMR (400MHz, d_6-DMSO) δ 1.1-1.7 (5H, m), 2.0-2.2 (1H, m), 2.5-3.0 (2H, m), 3.0-3.3 (1H, m), 3.8 (3H, s), 3.8-4.0 (1H, m), 4.3-4.8 (2.5H, m), 5.0-5.4 (3.5H, m), 6.8-7.0 (3H, m), 7.2-7.3 (1H, m), 7.7-8.6 (1H, m); ^{13}C NMR (100MHz, d_6-DMSO) δ 20.01, 20.29, 24.51, 24.64, 27.31 (3x CH_2), 32.89, 34.64, 34.71 (CH_2), 42.14 (CH_2), 47.40, 52.17, 52.37, 54.50 (2x CH), 55.36 (OCH_3), 66.51 (CH_2Ar), 81.42, 81.52, 83.28, 85.09 (CH_2F), 113.15, 113.51, 119.61, 119.74, 129.88 (ArCH), 138.71, 138.78, 156.71, 159.62 (ArC), 171.96, 172.08, 172.14, 173.33, 202.65, 202.80 (CO); ^{19}F NMR (376MHz, d_6-DMSO) δ -226.7, -226.8, -226.9, -230.2, -230.4, -230.4, -232.4, -232.6, -232.6.

[3S/R, (2S, α-R)]-5-Fluoro-3-(1-(α-trifluoromethyl benzyloxycarbonyl)-2-piperidinecarboxamido)-4-oxo-pentanoic acid (Example 9)



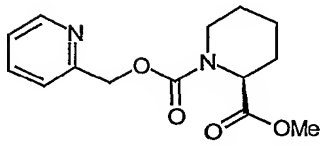
This was prepared from (R)-(-)-α-(trifluoromethyl)-benzyl alcohol using procedures similar to those described above in Methods F, and B-E to give an off-white solid (11mg, 54% last step): ^{1}H NMR (400MHz, d_6-DMSO) δ 1.1-1.8 (5H, m), 1.9-2.2 (1H, m), 2.3-3.0 (2H, m), 3.0-3.5 (1H, m), 3.8-4.2 (1H, m), 4.3-4.9 (2.5H, m), 5.0-5.3 (1.5H, m), 6.2-6.4 (1H, m), 7.4-7.6 (5H, m), 7.8-8.7 (1H, m); ^{19}F NMR (376MHz, d_6-DMSO) δ -75.7, -232.1, -232.5, -232.6, -232.7.

[3S/R, (2S)]-5-Fluoro-4-oxo-3-(1-(2-pyridinylmethoxycarbonyl)-2-piperidinecarboxamido)-pentanoic acid (Example 10)



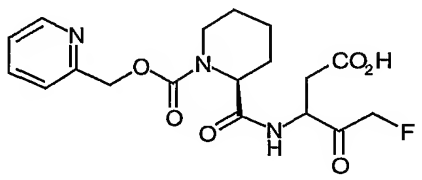
Method G:

(S)-1-(2-Pyridinylmethoxycarbonyl)-piperidine-2-carboxylic acid methyl ester



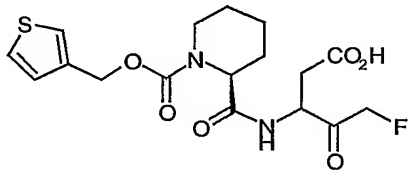
To a solution of 2-pyridylcarbinol (209μL, 2.17mmol) and dry THF (10mL) at 0°C under an atmosphere of nitrogen was added NaH (60% dispersion in mineral oil, 87mg, 2.17mmol), and the reaction mixture was stirred at 0°C for 30 mins. This mixture was then added dropwise to a solution of (S)-1-(chlorocarbonyl)-2-piperidinecarboxylic acid methyl ester (EP 75737) (425mg, 2.06mmol) and dry THF (10mL) at 0°C. The reaction mixture was allowed to stir for 1.5 h while warming to room temperature, then poured onto aq.$KHSO_3$ (25mL) and extracted with EtOAc (2 x 50mL). The combined organic phases were washed with aq.$NaHCO_3$, followed by brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by column chromatography (30% ethyl acetate in hexane) to give the title compound as a colourless oil (340mg, 59%): ^1H NMR (400MHz, $CDCl_3$) δ 1.3-1.8 (5H, m), 2.3 (1H, bs), 3.0 (0.5H, t, *J* 12.0Hz), 3.1 (0.5H, t, *J* 12.0Hz), 3.9 (3H, s), 4.2 (1H, m), 5.0 (1H, m), 5.3 (2H, dd, *J* 14.0Hz), 7.2-7.4 (2H, m), 7.7 (1H, m), 8.6 (1H, m)

[3S/R, (2S)]-5-Fluoro-4-oxo-3-(1-(2-pyridinylmethoxycarbonyl)-2-piperidinecarboxamido)-pentanoic acid (Example 10)



This was prepared from (S)-1-(2-pyridinylmethoxycarbonyl)-piperidine-2-carboxylic acid methyl ester using procedures similar to those described above in Methods B-E to give a clear glass (67mg, 100% last step): ^{1}H NMR (400MHz, d_6-DMSO) δ 1.23-1.61 (5H, m), 2.59 (1H, m), 2.82 (1H, m), 3.21 (1H, m), 3.95 (1H, m), 4.29-4.75 (3H, m), 5.09-5.21 (4H, m), 7.45-7.52 (2H, m), 7.94-8.59 (3H, m), 12.5 (1H, bs); ^{13}C NMR (100MHz, d_6-DMSO) δ 18.48 (CH_2), 22.97 (CH_2), 23.13 (CH_2), 25.73 (CH_2), 33.15 (CH_2), 40.74 (CH_2), 50.67, 50.94 (CH), 53.08 (CH), 64.85, 65.10 (CH_2), 81.85 (d, J 178Hz, CH_2F), 120.63 (CH), 122.27 (CH), 137.55 (CH), 146.61 (CH), 154.34 (C), 156.92, 157.28 (C), 170.56, 170.63 (C), 171.81 (C), 201.25 (CO); ^{19}F NMR (376MHz, d_6-DMSO) δ -75.21, -226.66, -226.70, -226.83, -226.87, -230.37, -232.38, -232.57, -232.62, -232.64.

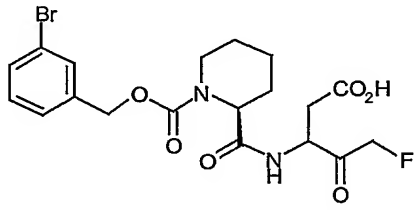
[3S/R, (2S)]-5-Fluoro-4-oxo-3-(1-(3-thienylmethoxycarbonyl)-2-piperidinecarboxamido)-pentanoic acid (Example 11)



This was prepared from 3-thiophenemethanol using procedures similar to those described above in Methods G, and B-E to give a white solid (27mg, 39% last step) after preparative HPLC: IR (solid) 3339, 2952, 1786, 1735, 1663cm^{-1}; ^{1}H NMR (400MHz, d_6-DMSO) δ 1.22-1.32 (2H, m), 1.41-1.57 (3H, m), 2.67 (1H, m), 2.71 (1H, m), 3.08 (1H, m), 3.87 (1H, m), 4.64-5.53 (5H, m), 7.08 (1H, m), 7.45-7.52 (2H, m), 8.44 (1H, m), 12.50 (1H, bs); ^{13}C NMR (100MHz, d_6-DMSO) δ 18.48, 18.58 (CH_2), 23.11 (CH_2), 25.87 (CH_2), 38.98 (CH_2), 52.71 (CH), 52.94 (CH), 60.87, 60.90 (CH_2), 122.22 (CH), 122.73 (CH), 126.07, 126.32 (CH), 136.50 (C), 170.23 (CO); No signal seen for AspCH_2, CH_2F or ketone CO, due to broadening of the signals; ^{19}F NMR (376MHz, d_6-DMSO) δ -226.62, -226.85, -226.90, -230.28, -232.37, -232.58, -232.69.

[3S/R, (2S)]-3-(1-(3-Bromobenzyloxycarbonyl)-2-piperidinecarboxamido)-5-fluoro-4-oxo-pentanoic acid

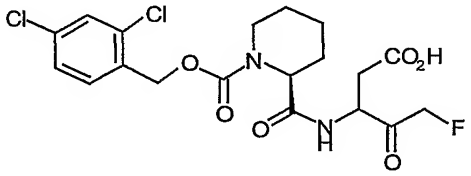
(Example 12)



This was prepared from 3-bromobenzyl alcohol using procedures similar to those described above in Methods G, and B-E to give a white solid (119mg, 58% last step): IR (solid) 1670, 1738, 1785cm^{-1}; ^{1}H NMR (400MHz, d_6-DMSO) δ 1.1-1.7 (5H, m), 2.0-2.2 (1H, m), 2.4-2.9 (2H, m), 2.9-3.3 (1H, m), 3.8-4.0 (1H, m), 4.2-4.8 (2.6H, m), 5.0-5.4 (3.4H, m), 7.2-7.4 (2H, m), 7.4-7.6 (2H, m), 7.7-8.7 (1H, m); ^{13}C NMR (100MHz, d_6-DMSO) δ 19.98, 20.26, 24.61, 27.09, 27.29, 27.66 (CH_2), 32.90, 34.71 (CH_2), 42.17 (CH_2), 47.40, 47.57, 52.19, 52.38, 54.32, 54.52 (2x CH), 65.80 (CH_2Ar), 81.54, 83.30, 85.10 (CH_2F), 121.96 (ArC), 126.54, 126.76, 130.28, 130.44, 130.98 (ArCH), 139.97, 140.00 (ArC), 156.05, 171.75, 172.08, 173.32, 202.78 (CO); ^{19}F NMR (376MHz, d_6-DMSO) δ -226.6, -226.8, -226.9, -230.1, -230.2, -230.3, -232.4, -232.5, -232.5, -232.6.

[3S/R, (2S)]-3-(1-(2,4-Dichlorobenzyloxycarbonyl)-2-piperidinecarboxamido)-5-fluoro-4-oxo-pentanoic acid

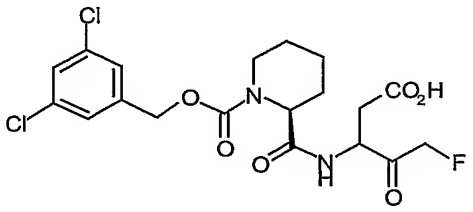
(Example 13)



This was prepared from 2,4-dichlorobenzyl alcohol using procedures similar to those described above in Methods G, and B-E to give a white solid (80mg, 64% last step): IR (solid) 1671, 1739, 1782cm^{-1}; ^{1}H NMR (400MHz, d_6-DMSO) δ 1.1-1.8 (5H, m), 2.0-2.2 (1H, m), 2.5-2.9 (2H, m), 3.0-3.3 (1H, m), 3.8-4.0 (1H, m), 4.2-4.8 (2.6H, m), 5.0-5.4 (3.4H, m), 7.4-7.6 (2H, m), 7.6-7.7 (1H, m), 7.7-8.6 (1H, m); ^{13}C NMR (100MHz, d_6-DMSO) δ 22.45, 26.96, 27.10, 29.75, 30.12 (CH_2), 35.39, 37.15 (CH_2), 44.70 (CH_2), 49.89, 50.07, 54.63, 54.89, 57.03 (2x CH), 66.10, 66.24 (CH_2Ar), 84.02, 85.62, 87.61 (CH_2F), 130.35, 131.71, 133.77 (ArCH), 136.15, 136.18, 136.26 (ArC), 174.07, 174.14, 174.34, 174.56, 174.62, 175.81, 205.13 (CO); ^{19}F NMR (376MHz, d_6-DMSO) δ -226.7, -226.8, -226.9, -227.2, -230.2, -230.3, -230.4, -232.4, -232.6, -232.6, -232.6.

[3S/R, (2S)]-3-(1-(3,5-Dichlorobenzyloxycarbonyl)-2-piperidinecarboxamido)-5-fluoro-4-oxo-pentanoic acid

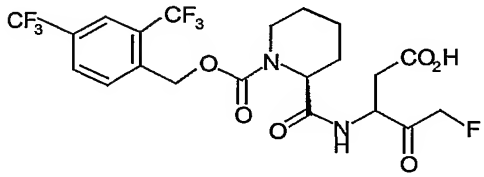
(Example 14)



This was prepared from 3,5-dichlorobenzyl alcohol using procedures similar to those described above in Methods G, and B-E to give a white solid (95mg, 52% last step): IR (solid) 1670, 1737, 1783cm^{-1}; ^{1}H NMR (400MHz, d_6-DMSO) δ 1.1-1.8 (5H, m), 2.0-2.2 (1H, m), 2.4-2.9 (2H, m), 3.0-3.3 (1H, m), 3.8-4.0 (1H, m), 4.3-4.8 (2.6H, m), 4.9-5.4 (3.4H, m), 7.3-7.5 (2H, m), 7.5-7.6 (1H, m), 7.7-8.6 (1H, m); ^{13}C NMR (100MHz, d_6-DMSO) δ; ^{19}F NMR (376MHz, d_6-DMSO) δ -226.6, -226.8, -226.9, -230.0, -230.2, -230.2, -230.2, -232.5, -232.5, -232.5, -232.6.

[3S/R, (2S)]-3-(1-(2,4-Bis(trifluoromethyl)benzyloxycarbonyl)-2-piperidinecarboxamido)-5-fluoro-4-oxo-pentanoic acid

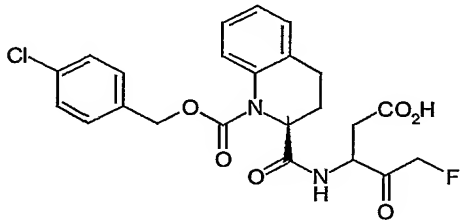
(Example 15)



This was prepared from 2,4-bis(trifluoromethyl)benzyl alcohol using procedures similar to those described above in Methods G, and B-E to give a white solid (59mg, 49% last step): IR (solid) 1655, 1684, 1735, 1772cm^{-1}; ^{1}H NMR

(400MHz, d_6-DMSO) δ 1.1-1.8 (5H, m), 2.0-2.2 (1H, m), 2.5-3.0 (2H, m), 3.0-3.3 (1H, m), 3.8-4.0 (1H, m), 4.2-4.8 (2.7H, m), 5.0-5.5 (3.3H, m), 7.7-8.0 (2H, m), 8.0-8.2 (1H, m), 8.2-8.6 (1H, m); ^{13}C NMR (100MHz, d_6-DMSO) δ; ^{19}F NMR (376MHz, d_6-DMSO) δ -59.88, -59.91, -61.80, -61.81, -61.84, -226.7, -226.8, -226.8, -226.9, -230.2, -230.5, -232.5, -232.7, -232.7, -232.7.

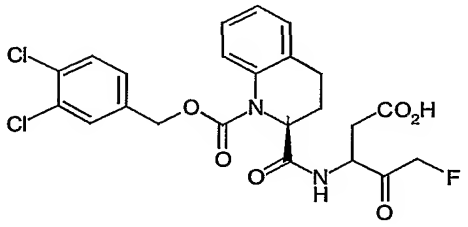
[3S/R, (2S)]-3-(1-(4-Chlorobenzyloxycarbonyl)-1,2,3,4-tetrahydro-quinolinyl-2-carboxamido)-5-fluoro-4-oxo-pentanoic acid (Example 16)



This was prepared from 4-chlorobenzyl alcohol and (S)-1-(chlorocarbonyl)-1,2,3,4-tetrahydro-quinoline-2-carboxylic acid methyl ester [prepared using a procedure similar to the one described for the synthesis of (S)-1-(chlorocarbonyl)-2-piperidinecarboxylic acid methyl ester in EP 75737] using procedures similar to those described in Methods G, and B-E to produce, after reverse phase HPLC, a white solid (57.4mg, 21%): IR (solid) 1794, 1694, 1522, 1384, 1317, 1203, 1045cm^{-1}; ^{1}H NMR (400MHz, d_6-DMSO) δ 1.59-1.78 (1H, m), 2.20-2.40 (1H, m), 2.40-2.82 (4H, m), 4.10-5.28 (6H, m), 6.90-7.20 (3H, m), 7.31-7.48 (4H, m), 7.60-7.77 (1H, m), 8.20-8.70 (1H, m); ^{13}C NMR (100MHz, d_6-DMSO) δ 25.53, 28.01, 34.80, 52.03, 57.85, 66.63, 83.12, 84.90, 126.41, 126.45, 127.86, 128.77, 129.85,

129.93, 132.93, 135.58, 137.22 172.01, 172.07, 172.20, 202.63, 202.77; ^{19}F NMR (376MHz, d_6-DMSO) δ -226.66, -226.93, -232.77 (bm), -232.91 (bm); Low Res MS ES+ 477.131, ES- 475.20.

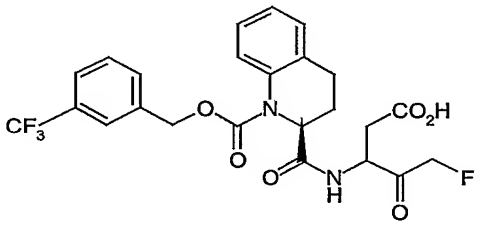
[3S/R, (2S)]-3-(1-(3,4-Dichlorobenzyloxycarbonyl)-1,2,3,4-tetrahydro-quinolinyl-2-carboxamido)-5-fluoro-4-oxo-pentanoic acid (Example 17)



This was prepared from 3,4-dichlorobenzyl alcohol and (S)-1-(chlorocarbonyl)-1,2,3,4-tetrahydro-quinoline-2-carboxylic acid methyl ester [prepared using a procedure similar to the one described for the synthesis of (S)-1-(chlorocarbonyl)-2-piperidinecarboxylic acid methyl ester described in EP 75737] using procedures similar to those described in Methods G, and B-E to produce, after reverse phase HPLC, a white solid (131.5mg, 35%): IR (solid) 1693, 1527, 1374, 1331, 1198, 1055, 1026cm^{-1}; ^1H NMR (400MHz, d_6-DMSO) δ 1.58-1.79 (1H, m), 2.19-2.39 (1H, m), 2.40-2.83 (4H, m), 4.00-5.25 (6H, m), 6.92-7.02 (1H, m), 7.05-7.21 (2H, m), 7.30-7.40 (1H, m), 7.55-7.78 (3H, m), 8.21-8.75 (1H, m); ^{13}CNMR (100MHz, d_6-DMSO) δ 24.11, 24.21, 26.58, 26.78, 26.96, 31.68, 33.18, 33.37, 50.63, 50.92, 56.41, 56.49, 56.74, 64.30, 64.52, 122.45, 124.96, 125.01, 126.37, 126.44, 126.69, 126.76, 128.44, 128.52, 129.50, 129.45, 129.99, 130.32, 135.74, 135.92, 136.29,

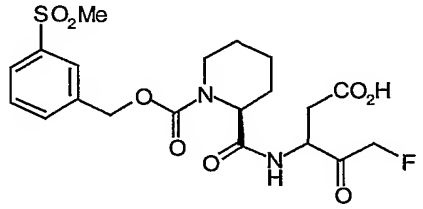
136.23, 152.81, 170.62, 170.65, 170.69, 170.79, 171.60, 201.19, 201.33; ^{19}F NMR (376MHz, d_6-DMSO) δ -226.75, -227.01, -232.90 (bm).

[3S/R, (2S)]-3-(1-(3-Trifluoromethylbenzyloxycarbonyl)-1,2,3,4-tetrahydro-quinolinyl-2-carboxamido)-5-fluoro-4-oxo-pentanoic acid (Example 18)



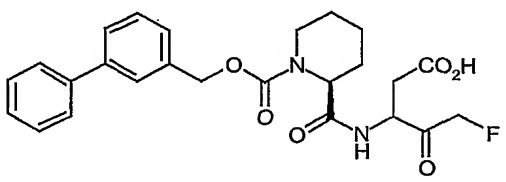
This was prepared from 3-trifluoromethylbenzyl alcohol and (S)-1-(chlorocarbonyl)-1,2,3,4-tetrahydro-quinoline-2-carboxylic acid methyl ester [prepared using a procedure similar to the one described for the synthesis of (S)-1-(chlorocarbonyl)-2-piperidinecarboxylic acid methyl ester in EP 75737] using procedures similar to those described above in Methods G, and B-E to produce, after reverse phase HPLC, a white foam (69.2mg, 30%): IR (solid) 1689, 1527, 1393, 1327, 1203, 1165, 1122cm^{-1}; ^{1}H NMR (400MHz, d_6-DMSO) δ 1.60-1.79 (1H, m), 2.20-2.39 (1H, m), 2.40-2.80 (4H, m), 4.10-5.37 (6H, m), 6.92-7.19 (3H, m), 7.51-7.76 (4H, m), 8.19-8.74 (1H, m); ^{13}C NMR (100MHz, d_6-DMSO) □ 25.52, 25.63, 27.99, 28.20, 34.55, 34.77, 52.30, 57.84, 57.90, 66.61, 83.05, 83.27, 84.83, 85.04, 123.90, 124.31, 124.35, 124.91, 126.36, 127.75, 129.69, 131.81, 131.88, 137.17, 138.02, 138.06, 154.33, 172.02, 172.06, 172.18, 202.61, 202.75; ^{19}FNMR (376MHz, d_6-DMSO) δ -226.84, -227.08, -232.94 (bm).

[3S/R, (2S)]-5-Fluoro-3-(1-(3-methylsulphonylbenzyloxycarbonyl)-2-piperidinecarboxamido)-4-oxo-pentanoic acid (Example 19)



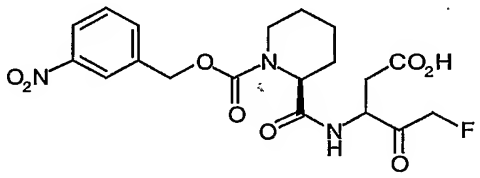
This was prepared from 3-methylsulfonylbenzyl alcohol using procedures similar to those described above in Methods G, and B-E to give a off-white solid (51.7mg, 41% last step): IR (solid) 1676, 1733, 1787cm^{-1}; ^1H NMR (400MHz, d_6-DMSO) δ 1.1-1.7 (5H, m), 2.0-2.2 (1H, m), 2.4-2.9 (3H, m), 3.1-3.3 (3H, s), 3.8-4.0 (1H, m), 4.2-4.8 (2.6H, m), 5.0-5.4 (3.4H, m), 7.6-8.0(4H, m), 8.0-8.7(1H, m); ^{13}C NMR (100MHz, d_6-DMSO) δ 19.99, 24.61, 27.26, 27.65, 29.36(CH_2), 32.86, 34.69 (CH_2), 42.20(CH_2), 43.77(CH_3), 47.41, 47.59, 52.39, 54.50 (2 x CH), 65.73 (CH_2), 81.53, 83.30, 85.10 (CH_2), 125.84, 126.64, 129.99, 132.69 (ArCH), 138.89, 141.31(ArC), 171.68, 172.07, 172.13, 173.34, 202.65, 202.79(CO); ^{19}F NMR (376MHz, d_6-DMSO) δ -226.6, -226.8, -230.2, -232.4, -232.4, -232.5, -232.6.

[3S/R, (2S)]-5-Fluoro-4-oxo-3-(1-(3-phenylbenzyloxycarbonyl)-2-piperidinecarboxamido)-pentanoic acid (Example 20)



This was prepared from 3-phenylbenzyl alcohol using procedures similar to those described above in Methods G, and B-E to give a white powder (105.3mg, 46% last step): IR (solid) 1671, 1739cm^{-1}; ^{1}H NMR (400MHz, d_6-DMSO) δ 1.1-1.7 (5H, m), 2.0-2.2 (1H, m), 2.5-2.8 (2H, m), 3.0-3.3 (1H, m), 3.9-4.0 (1H, m), 4.2-4.8 (2.5H, m), 5.0-5.4 (3.5H, m), 7.2-7.7 (9H, m), 7.8-8.7 (1H, m); ^{13}C NMR (100MHz, d_6-DMSO) δ 20.53, 25.14, 27.63, 27.83 (CH_2), 33.32, 35.23 (CH_2), 42.68 (CH_2), 47.90, 48.07, 52.70, 52.89, 55.06 (2 x CH), 67.20 (CH_2), 83.80, 85.61 (CH_2), 126.69, 127.04, 127.37, 127.60, 128.43, 129.82, 129.95, 131.51 (ArCH), 138.39, 138.41, 140.76, 141.18 (ArC), 172.34, 172.47, 172.59, 172.64, 203.26 (CO); ^{19}F NMR (376MHz, d_6-DMSO) δ -226.7, -226.8, -230.2, -230.3, -232.3, -232.5, -232.5, -232.6.

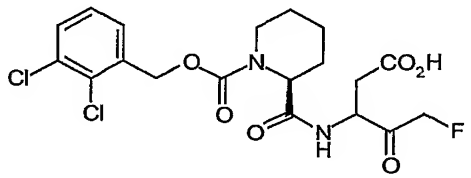
[3S/R, (2S)]-5-Fluoro-3-(1-(3-nitrobenzyloxycarbonyl)-2-piperidinecarboxamido)-4-oxo-pentanoic acid (Example 21)



This was prepared from 3-nitrobenzyl alcohol using procedures similar to those described above in Methods G, and B-E to give a off-white solid (35.6mg, 58% last step): IR (solid) 1671, 1739, 1786cm^{-1}; ^{1}H NMR (400MHz, d_6-DMSO) δ 1.1-1.7 (5H, m), 2.0-2.2 (1H, m), 2.5-2.9 (2H, m), 3.0-3.3 (1H, m), 3.8-4.0 (1H, m), 4.2-4.8 (2.5H, m), 5.0-5.4 (3.5H, m), 7.6-7.9 (2H, m), 8.1-8.3 (2H, m), 8.4-8.6 (1H, m); ^{13}C NMR (100MHz, d_6-DMSO) δ 19.97, 24.46, 27.25, 27.63 (CH_2), 34.62 (CH_2), 42.20 (CH_2), 47.42,

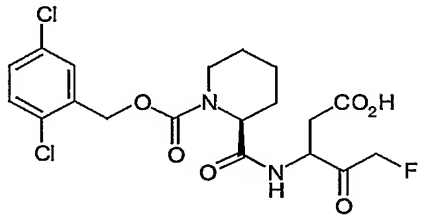
52.16, 52.40, 52.51, 54.55 (2 x CH), 65.47 (CH_2), 83.29, 85.09 (CH_2), 122.26, 122.30, 122.33, 123.06, 130.40, 134.29 (ArCH), 139.51, 139.55, 148.12 (ArC), 172.07 (CO); ^{19}F NMR (376MHz, d_6-DMSO) δ -226.7, -226.8, -226.9, -230.2, -230.3, -232.4, -232.6, -232.6, -232.6.

[3S/R, (2S)]-5-Fluoro-3-(1-(2,3-dichlorobenzyloxycarbonyl)-2-piperidinecarboxamido)-4-oxo-pentanoic acid (Example 22)



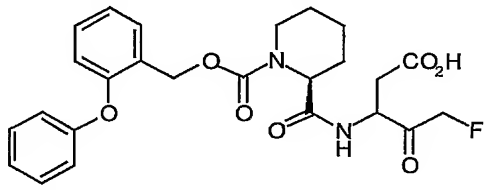
This was prepared from 2,3-dichlorobenzyl alcohol using procedures similar to those described above in Methods G, and B-E to give a off-white solid (161.2mg, 83% last step): IR (solid) 1670, 1716, 1739, 1781cm^{-1}; ^1H NMR (400MHz, d_6-DMSO) δ 1.1-1.8 (5H, m), 2.0-2.2 (1H, m), 2.5-2.9 (2H, m), 3.0-3.3 (1H, m), 3.8-4.0 (1H, m), 4.2-4.8 (2.5H, m), 5.0-5.4 (3.5H, m), 7.3-7.5 (2H, m), 7.6-7.7 (1H, m), 7.8-8.7 (1H, m); ^{13}C NMR (100MHz, d_6-DMSO) δ 20.48, 24.97, 25.14, 27.84, 28.18 (CH_2), 33.41, 35.20 (CH_2), 47.90, 48.07, 52.66, 52.90, 54.86, 55.07 (2 x CH), 64.69, 65.04 (CH_2), 82.04, 83.83, 85.62 (CH_2), 128.39, 128.69, 129.19, 130.87 (ArCH), 132.67, 137.66, 137.69, 137.73, 155.58, 156.29 (ArC), 172.17, 172.36, 172.57, 172.63, 173.82, 203.28 (CO); ^{19}F NMR (376MHz, d_6-DMSO) δ -226.7, -226.8, -226.9, -230.2, -230.3, -230.4, -232.4, -232.5, -232.6, -232.6, -232.7.

[3S/R, (2S)]-5-Fluoro-3-(1-(2,5-dichlorobenzyloxycarbonyl)-2-piperidinecarboxamido)-4-oxo-pentanoic acid (Example 23)



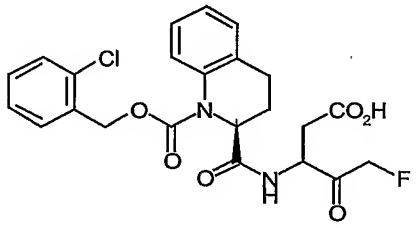
This was prepared from 2,5-dichlorobenzyl alcohol using procedures similar to those described above in Methods G, and B-E to give a pale yellow solid (114.6mg, 71% last step): IR (solid) 1670, 1739, 1782cm^{-1}; ^{1}H NMR (400MHz, d_6-DMSO) δ 1.1-1.8 (5H, m), 2.0-2.2 (1H, m), 2.4-2.9 (2H, m), 3.0-3.4 (1H, m), 3.8-4.0 (1H, m), 4.3-4.8 (2.5H, m), 5.0-5.4 (3.5H, m), 7.3-7.6 (3H, m), 7.7-8.7 (1H, m); ^{13}C NMR (100MHz, d_6-DMSO) δ 20.48, 20.77, 24.97, 25.12, 27.61, 27.83, 29.89 (CH_2), 33.42, 33.50, 35.21 (CH_2), 42.73 (CH_2), 47.92, 48.11, 52.71, 52.67, 53.34, 54.88, 55.01, 55.13 (2 x CH), 64.15, 64.30 (CH_2), 82.06, 83.81, 85.61 (CH_2), 129.46, 129.83, 130.05, 130.38, 131.80, 131.92 (ArCH), 132.78, 137.16, 137.19 (ArC), 171.65, 172.15, 172.35, 172.59, 173.83, 203.29 (CO); ^{19}F NMR (376MHz, d_6-DMSO) δ -226.7, -226.8, -226.9, -230.1, -230.3, -232.4, -232.5, -232.5, -232.6.

[3S/R, (2S)]-5-Fluoro-4-oxo-3-(1-(2-phenoxybenzyloxycarbonyl)-2-piperidinecarboxamido)-pentanoic acid (Example 24)



This was prepared from 3-phenoxybenzyl alcohol using procedures similar to those described above in Methods G, and B-E to give, after reverse phase HPLC, a white powder (20.0mg, 35% last step): ^{1}H NMR (400MHz, d_6-DMSO) δ 1.19-1.52 (5H, m), 2.06 (1H, t), 2.56 (1H, m), 2.78 (1H, m), 2.99 (1H, m), 3.73 (1H, m), 4.29-5.20 (6H, m), 7.10-7.48 (9H, m), 8.10, 8.50 (1H, 2 x d, J 8.0Hz); ^{13}C NMR (100MHz, d_6-DMSO) δ 20.01, 21.52 (CH_2), 24.57 (CH_2), 27.22, 27.60 (CH_2), 34.70 (CH_2), 41.98 (CH_2), 52.16, 53.38 (CH), 54.51, 56.05 (CH), 62.32 (CH_2), 83.30 (d, J 178Hz, CH_2), 114.25 (C), 117.13 (C), 118.06 (CH), 119.38, 119.63 (CH), 123.51 (CH), 124.38 (CH), 128.24, 128.27 (C), 129.94, 130.15, 130.35 (CH), 171.63, 171.89 (CO), 172.07, 172.13 (CO); ^{19}F NMR (376MHz, d_6-DMSO) δ-226.67, -226.80, -232.43, -232.57, -232.63.

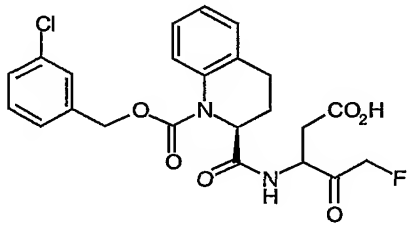
[3S/R, (2S)]-3-(1-(2-Chlorobenzyloxycarbonyl)-1,2,3,4-tetrahydro-quinolinyl-2-carboxamido)-5-fluoro-4-oxo-pentanoic acid (Example 25)



This was prepared from 2-chlorobenzyl alcohol and (S)-1-(chlorocarbonyl)-1,2,3,4-tetrahydro-quinoline-2-carboxylic acid methyl ester [prepared using a procedure similar to the one described for the synthesis of (S)-1-(chlorocarbonyl)-2-piperidinecarboxylic acid methyl ester described in EP 75737] using procedures similar to those described in Methods G, and B-E to leave a colourless

solid (64mg, 99.3% last step): IR (solid) 1789.2, 1694.6, 1527.6, 1493.0, 1392.2, 1324.1, 1282.5, 1235.7, 1207.9, 1172.1, 1121.6, 1048.5, 757.3.; ^{1}H NMR (400MHz, d_6-DMSO) δ 1.75 (1H, m), 2.30 (1H, m), 2.44-2.88 (4H, m), 4.15-5.35 (6H, m), 7.00 (1H, m), 7.17 (2H, m), 7.39 (2H, m), 7.49 (2H, m), 7.70 (1H, m), 8.28+8.68 (1H, 2xm); ^{13}C NMR (100MHz, d_6-DMSO) □ 24.00, 24.13, 26.47, 26.71, 26.89, 33.12, 33.31 (CH2), 45.84, 50.82, 56.56 (CH), 63.12, 63.24, 63.36 (CH2), 82.59 (d, J 177, CH2F), 122.19, 122.68, 125.00, 128.76, 128.86, (CH), 130.22, 131.13, 132.35, 132.38135.64, 135.83 (C), 152.70, 170.40, 170.43, 170.55, 170.59 (C=O), 200.97, 202.10, 201.24 (FCH2C=O).); ^{19}F NMR (376MHz, d_6-DMSO) □□□-226.63 (t, J 45), -226.92 (t, J 45), -230.52 (t, J 45), -232.84 (m).

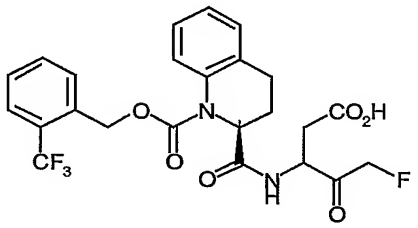
[3S/R, (2S)]-3-(1-(3-Chlorobenzyloxycarbonyl)-1,2,3,4-tetrahydro-quinolinyl-2-carboxamido)-5-fluoro-4-oxo-pentanoic acid (Example 26)



This was prepared from 3-chlorobenzyl alcohol and (S)-1-(chlorocarbonyl)-1,2,3,4-tetrahydro-quinoline-2-carboxylic acid methyl ester [prepared using a procedure similar to the one described for the synthesis of (S)-1-(chlorocarbonyl)-2-piperidinecarboxylic acid methyl ester described in EP 75737] using procedures similar to those described in Methods G, and B-E to produce a colourless solid (124mg, 99.3% last step): IR (solid) 1784.4, 1694.3, 1576.7, 1530.4, 1492.9, 1388.3, 1328.7, 1209.4,

1171.1, 1121.3, 1052.5, 938.5, 768.0.; ^{1}H NMR (400MHz, d_6-DMSO) δ 1.70 (1H, m), 2.33 (1H, m), 2.40-2.85 (4H, m), 4.05-5.30 (6H, m), 7.02 (1H, m), 7.18 (2H, m), 7.40 (4H, m), 7.71 (1H, m), 8.28+8.70(1H, 2xm); ^{13}C NMR δ (100MHz, d_6-DMSO)25.55, 25.67, 25.76, 28.02, 28.24, 28.33,33.04, 33.10, 34.63, 34.82 (CH2), 47.35, 52.06, 52.30, 57.82, 57.89 (CH), 84.11 (d, J 177, CH2F), 123.90, 124.14, 126.40, 126.45, 126.53, 127.63, 127.67, 127.73, 127.79, 128.23, 130.67 (CH), 133.41, 137.19, 139.05, 139.10 (C), 154.19, 154.31, 172.03, 172.07, 172.13, 173.06, 173.20 (C=O), 202.49, 202.63, 202.76 (FCH2C=O); ^{19}F NMR (376MHz, d_6-DMSO) □□□-226.62 (t, J 45), -226.91 (t, J 45), -232.73 (br m).

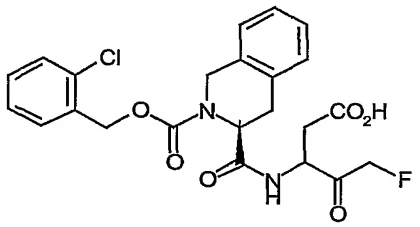
[3S/R, (2S)]-3-(1-(2-trifluoro methylbenzyloxycarbonyl)-1,2,3,4-tetrahydro-quinolinyl-2-carboxamido)-5-fluoro-4-oxo-pentanoic acid (Example 27)



This was prepared from 2-trifluoromethylbenzyl alcohol and (S)-1-(chlorocarbonyl)-1,2,3,4-tetrahydro-quinoline-2-carboxylic acid methyl ester [prepared using a procedure similar to the one described for the synthesis of (S)-1-(chlorocarbonyl)-2-piperidinecarboxylic acid methyl ester described in EP 75737] using procedures similar to those described in Methods G, and B-E to produce a colourless solid (101mg, 95.1% last step): IR (solid) 3308, 1694.4, 1527.1, 1493.3, 1456.7, 1398.4,

1314.9, 1209.7, 1168.5, 1118.3, 1052.8, 1039.1, 768.0 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.99-3.20 (5H, m), 3.35-5.20 (5.5H, m), 5.30-5.50 (1.5H, m), 6.95-7.35 (6H, m), 7.44-7.78 (3H, m); ^{13}C (100 MHz, d_6-DMSO) δ 25.63, 27.96, 34.80 (CH_2), 51.98, 57.81 (CH), 63.70 (CH_2), 84.08 (d, CH_2F, J 176), 124.58 (q, CF_3, J 272), 123.99, 126.29, 126.41, 126.46, 127.81, 127.94, 128.99, 129.02, 130.14 (CH), 134.55, 137.14 (C), 154.13, 171.88, 172.07, 172.12, 202.46, 202.59, 202.72 (C=O); ^{19}F NMR (376 MHz, $CDCl_3$) -60.17 (s), -230.69 (t, J 48), -231.64 (t, J 48), -231.82 (t, J 48), -232.38 (t, J 48), -232.82 (t, J 48).

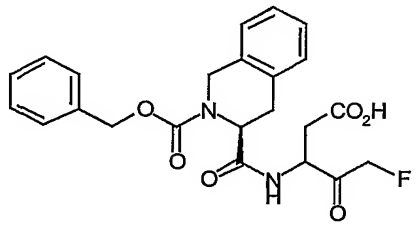
[3S/R, (2S)]-3-(1-(2-Chlorobenzyloxycarbonyl)-1,2,3,4-tetrahydro-isoquinolinyl-2-carboxamido)-5-fluoro-4-oxo-pentanoic acid (Example 28)



This was prepared from 2-chlorobenzyl alcohol and (S)-1-(chlorocarbonyl)-1,2,3,4-tetrahydro-isoquinoline-2-carboxylic acid methyl ester [prepared using a procedure similar to the one described for the synthesis of (S)-1-(chlorocarbonyl)-2-piperidinecarboxylic acid methyl ester described in EP 75737] using procedures similar to those described in Methods G, and B-E to produce, after reverse phase HPLC, a white foam (57.9mg, 98% last step): IR (solid) 1793, 1680, 1516, 1404, 1337, 1209, 1122, 1055; ^1H NMR (400 MHz, d6-DMSO) δ 2.20- 2.80 (2H, m, Asp), 3.00-3.21 (2H, m, CHCH2), 3.84-5.30 (8H, m, NCH2, OCH2, CH2F,

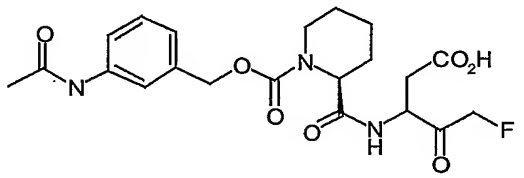
2xHa), 7.08-7.60 (8H, M, ArH), 8.08-8.63 (1H, m, NH); ^{13}C NMR (100MHz, d_6-DMSO) δ 30.40, 30.70, 30.85, 33.10, 33.23 (CH2, CHCH2, AspCH2), 43.59, 43.71 (CH2, NCH2), 51.02, 53.17, 53.50, 53.60 (CH, Has), 62.83, 62.91, 63.05 (CH2, OCH2), 81.18, 81.39, 82.96 (CH2, CH2F), 125.86, 126.28, 126.46, 126.66, 128.12, 128.33, 128.67, (CH, ArCH), 131.65, 131.89, 132.02, 132.45, 132.90 (C, ArC), 170.29, 170.60 (C, C=O), 200.81, 200.93 (C, C=O); ^{19}F NMR (376MHz, d_6-DMSO) δ -226.80, -226.96, -227.04, -232.81, -233.10, -233.29, -233.41.

[3S/R, (2S)]-3-(1-(Benzyloxycarbonyl)-1,2,3,4-tetrahydro-isoquinolinyl-2-carboxamido)-5-fluoro-4-oxo-pentanoic acid (Example 29)



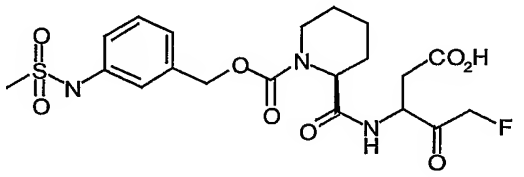
This was prepared from (S)-1-(benzyloxycarbonyl)-1,2,3,4-tetrahydro-isoquinoline-2-carboxylic acid [J. Med. Chem., (1991), 3350] using procedures similar to those described in Methods C-E to produce title compound as a yellow gum (153mg, 100% last step): IR (solid) 1736, 1360, 1231, 1217; ^{1}H NMR (400 MHz, d6-DMSO) 2.24-2.40 (1H, m), 2.57-2.69 (1H, m), 3.05-3.17 (2H, m), 4.14-4.84 (6H, m), 5.07-5.21 (2H, m), 7.20-7.44 (9H, m), 8.49-8.56 (1H, m), 12.41 (1H, br s); ^{19}F NMR (376MHz, d_6-DMSO) δ -226.7, -226.969, -227.0, -233.0, -233.0, -233.2, -233.3.

[3S/R, (2S)]-5-Fluoro-3-(1-(3-acetamidobenzyloxycarbonyl))-2-piperidinecarboxamido)-4-oxo-pentanoic acid (Example 30)



This was prepared from N-(3-hydroxy methylphenyl)acetamide using procedures similar to those described above in Methods G, and B-E to give white solid (26.9mg, 99.2% last step): IR (solid) 1666.3, 1786.9 cm-1; ^{1}H NMR (400MHz, d_6-DMSO) δ 1.1-1.8(5H, m, pip), 1.9-2.2(4H, m, Ac, pip), 2.4-2.9(2H, m, Asp), 3.0-3.6(1H, m, pip), 3.8-4.0(1H, m, pip), 4.2-5.5(6H, m, Asp, pip, -CH2-, -CH2-F), 6.9-7.1(1H, m, Ar), 7.2-7.3(1H, m, Ar), 7.4-7.6(2H, m, Ar), 7.8-8.7(1H, m, NH), 9.9-10.1(1H, br s, NH); ^{13}C NMR (100MHz, d_6-DMSO) δ 20.02(CH2, pip), 24.35(CH3, Ac), 24.65, 27.33, 42.11(CH2, pip), 54.27, 54.52(CH, Asp, pip), 66.70(CH2, -CH2-Ar), 118.18, 118.74, 122.34, 129.10(CH, Ar), 137.62, 139.79(C, Ar), 168.71(C, C=O); ^{19}F NMR (376MHz, d_6-DMSO) δ -226.7, -232.5

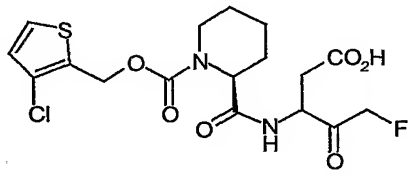
[3S/R, (2S)]-5-Fluoro-3-(1-(3-methanesulfonamido) benzyloxycarbonyl)-2-piperidinecarboxamido)-4-oxo-pentanoic acid (Example 31)



This was prepared from N-(3-hydroxy methyl-phenyl)methanesulfonamide using procedures similar to

those described above in Methods G, and B-E to give white solid (35.2mg, 98.7% last step): IR (solid) 1668.0, 1738.4 cm-1; ^{1}H NMR (400MHz, d_6-DMSO) δ 1.1-1.7(5H, m, pip), 2.0-2.2(1H, m, pip), 2.5-2.9(2H, m, Asp), 2.9-3.5(4H, m, pip, -SO2Me), 3.8-4.0(1H, m, pip), 4.5-5.5(6H, m, Asp, pip, -CH2-, -CH2-F), 7.0-7.4(4H, m, Ar), 8.0-8.8(1H, br s, NH), 9.6-10.0(1H, br s, NH) ; ^{13}C NMR (100MHz, d_6-DMSO) δ 20.03, 20.14, 24.66, 27.17, 27.32(CH2, pip), 39.60(CH3, -SO2Me), 42.11(CH2, pip), 54.28, 54.46(CH, Asp, pip), 66.34(CH2, -CH2-Ar), 118.44, 119.14, 122.81, 129.74(CH, Ar), 138.48, 138.92(C, Ar), 171.56(C, C=O) ; ^{19}F NMR (376MHz, d_6-DMSO) δ -232.5

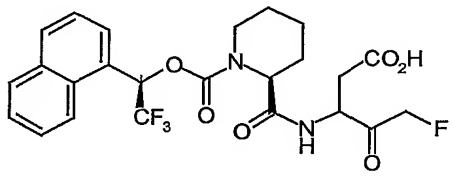
[3S/R, (2S)]-5-Fluoro-4-oxo-3-(1-(3-chloro-2-thienylmethoxycarbonyl)-2-piperidinecarboxamido)-pentanoic acid (Example 32)



This was prepared from 3-chloro-2-thiophenemethanol using procedures similar to those described above in Methods G, and B-E to give a pale cream solid (4.4mg, 98.7% last step): IR (Nicolet Avantar, 360 Omni Sampler, cm-1) 3316, 2951, 1677; ^{1}H NMR (400MHz, d_6-DMSO) δ 1.21-1.75 (5H, m, CH2pip), 2.07 (1H, m CH2pip), 2.67 (1H, m, CH2pip), 2.82-3.13 (2H, m, CH2Asp), 3.86 (1H, m, CH2), 4.57-5.26 (6H, m), 7.07 (1H, s, CHthiophene), 7.69 (1H, s, CHthiophene), 8.44 (1H, d, J 7, NH) ; ^{13}C NMR (100MHz, d_6-DMSO) δ 19.92 (CH2), 24.46 (CH2), 27.22 (CH2), 34.67 (CH2), 39.13 (CH2), 42.15 (CH2), 52.16, 52.43 (CH, a-

CH), 54.53 (CH, a-CH), 59.01 (CH2), 83.25 (d, J 178, CH2F), 124.27 (C), 127.65 (CH), 131.75 (C), 171.56 171.81 (C, CO), 172.07, 172.14 (C, CO); ^{19}F NMR (376MHz, d_6-DMSO) δ -226.90,- 232.39, -232.62, -232.69.

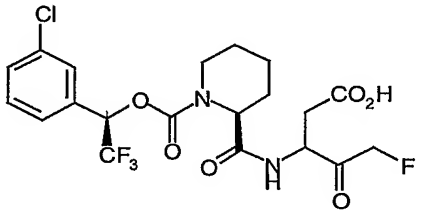
2-(1-Carboxymethyl-3-fluoro-2-oxo-propylcarbamoyl)-piperidine-1-carboxylic acid 2,2,2-trifluoro-1-naphthalen-1-yl-ethyl ester (Example 33)



This was prepared from (R)-(-)-α-(trifluoromethyl)-naphthyl alcohol (prepared according to *Tetrahedron*, 1993, **49**(9), 1725-1738) using procedures similar to those described above in Methods F, and B-E to give a white solid (176.2mg, 98.1% last step): IR (Solid) 1712.7, 1785.8 cm-1; ^{1}H NMR(400MHz, d_6-DMSO) δ 1.1-1.9(5H, m, pip), 1.9-2.3(1H, m, pip), 2.5-2.9(2H, m, Asp), 3.0-3.6(1H, m, pip), 4.1-4.2(1H, m, pip), 4.3-5.3(4H, m, Asp, pip, -CH2-F), 7.0-7.1(1H, m, Ar-CH-), 7.5-8.4(7H, m, Ar), 8.4-8.8(1H, m, NH)); ^{13}C NMR (100MHz, d_6-DMSO) δ 20.31, 24.86, 25.16, 27.97, 28.25(CH2, pip), 35.22(CH2, Asp), 43.04(CH2, pip), 52.68, 52.87(CH, Asp), 55.02, 55.21(CH, pip), 69.27, 69.57, 69.92(C, CF3), 83.88, 83.96, 85.65, 85.74(CH2, -CH2-F), 123.35(C, Ar), 124.01, 124.18, 126.09, 126.24, 126.79, 126.97, 127.09, 127.28, 127.95(CH, Ar), 128.44, 128.55, 128.94(C, Ar), 129.59, 130.94, 131.01, 131.12(C, Ar), 131.44, 131.52, 133.93, 134.06(C, Ar), 153.71, 154.40, 171.73, 171.87, 171.96, 172.54, 203.20, 203.34(C, C=O); ^{19}F NMR (376MHz, d_6-DMSO)

δ -74.5, -74.6, -74.6, -74.6, -74.8, -74.9, -75.3, -226.5, -226.6, -226.8, -227.0, -230.0, -230.1, -230.2, -232.1, -232.5, -232.5, -232.6.

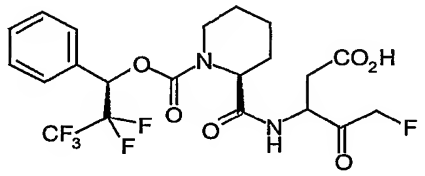
[3S/R, (2S,α-R)]-5-Fluoro-3-(1-(α-trifluoromethyl(3-chloro benzyloxycarbonyl)-2-piperidinecarboxamido)-4-oxo-pentanoic acid (Example 34)



This was prepared from (R)-(-)-α- (trifluoromethyl)- 3 chlorobenzyl alcohol (prepared using procedures from *Tetrahedron*, 1993, **49**(9), 1725-1738)using procedures similar to those described above in Methods F, and B-E to give a white solid (101mg, 99%): IR (Solid) 1716.1, 1782.8 cm-1; 1H NMR (400MHz, DMSO) δ 1.1-1.8(5H, m, pip), 1.9-2.3(1H, m, pip), 2.4-2.9(2H, m, Asp), 3.0-3.5(1H, m, pip), 3.8-4.1(1H, m, pip), 4.3-5.3(4H, m, Asp, pip, -CH2-F), 6.3-6.5(1H, m, Ar-CH-), 7.3-7.7(4H, m, Ar), 7.7-8.8(1H, m, NH) ; ^{13}C NMR (100MHz, d_6-DMSO) δ 18.25, 18.34, 22.79, 23.08, 25.98, 26.06, 26.22(CH2, pip), 33.20, 33.26(CH2, Asp), 41.03(CH2, pip), 45.90, 46.03, 46.29, 50.65, 50.76, 50.92(CH, Asp), 53.07, 53.12, 53.21, 53.27, 53.44, 53.50(CH, pip), 69.87, 69.96, 70.19, 70.52(CH, CF3), 81.49, 81.85, 83.63(CH2, -CH2-F), 117.94, 120.74, 123.53(C, Ar), 125.09, 125.27, 126.35, 126.57, 128.51, 128.57, 128.68, 129.28, 129.33, 129.66(CH, Ar), 132.08, 132.18, 132.64, 132.72, 133.01(C, Ar), 151.54, 151.60, 151.68, 151.93, 152.30, 169.51, 169.72, 169.99, 170.05, 170.17, 170.49, 170.54, 170.61, 171.76, 201.02, 201.16,

201.30(C, C=O); ^{19}F NMR (376MHz, d_6-DMSO) δ -75.4, -75.4, -75.5, -75.6, -75.7, -75.7, -75.7, -75.8, -75.8, -226.6, -226.7, -226.8, -227.0, -230.0, -230.0, -230.1, -232.2, -232.5, -232.6.

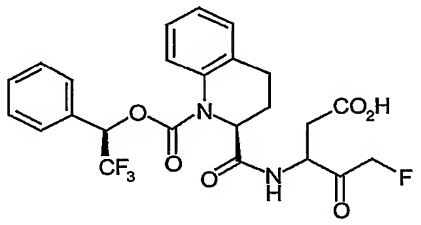
[3S/R, (2S,α-R)]-5-Fluoro-3-(1-(α-pentafluoromethyl (benzyloxycarbonyl)-2-piperidinecarboxamido)-4-oxo-pentanoic acid (Example 35)



This was prepared from (R)-(-)-α- (pentafluoromethyl)-benzyl alcohol (prepared according to *Tetrahedron*, 1993, **49**(9), 1725-1738) using procedures similar to those described above in Methods F, and B-E to give a white solid (59.7mg, 99.2%); IR (Solid) 1721.1, 1736.7 cm-1; 1H NMR (400MHz, DMSO) δ 1.1-1.8(5H, m, pip), 1.9-2.3(1H, m, pip), 2.5-2.9(2H, m, Asp), 3.0-3.5(1H, m, pip), 3.7-4.2(1H, m, pip), 4.3-5.3(4H, m, Asp, pip, -CH2-F), 6.2-6.4(1H, m, Ar-CH-), 7.3-7.6(5H, m, Ar), 7.7-8.8(1H, m, NH) ; ^{13}C NMR (100MHz, d_6-DMSO) δ 18.14, 18.39, 22.88, 22.99, 26.05, 26.19(CH2, pip), 33.11, 33.22(CH2, Asp), 40.91, 40.95(CH2, pip), 45.85, 46.08, 46.22, 50.57, 50.64, 50.91, 50.98(CH, Asp), 52.97, 53.13, 53.31(CH, pip), 69.30, 69.39, 69.51, 69.60, 69.70, 69.90, 70.26(CH, C2F5), 79.77, 80.13, 81.52, 81.90, 83.59, 83.72(CH2, -CH2-F), 126.96, 127.08, 127.27, 127.30, 127.54, 128.62, 128.72(CH, Ar), 129.56, 129.86(C, Ar), 151.26, 151.35, 151.61, 152.26, 169.52, 169.65, 169.81, 170.17, 170.22,

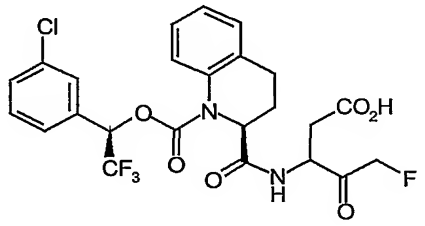
170.52, 170.63, 171.66, 171.75, 201.17, 201.31(C, C=O); ^{19}F NMR (376MHz, d_6-DMSO) δ -81.1, -81.2, -81.2, -81.2, -81.3, -81.3, -81.3, -118.4 to -118.6, -119.1 to -119.3, -126.0 to -126.6, -127.0 to -127.4, -226.6, -226.8, -226.9, -227.0, -230.0, -230.2, -230.4, -232.0, -232.6, -232.8.

[3S/R, (2S,α-R)]-5-Fluoro-3-(1-(α-trifluoromethyl benzyloxycarbonyl-1,2,3,4-tetrahydro-quinolinyl-2-carboxamido)-4-oxo-pentanoic acid (Example 36)



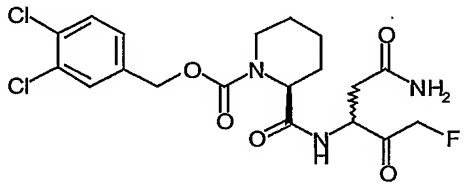
This was prepared from (R)-(-)-a- (trifluoromethyl)-benzyl alcohol using procedures similar to those described above in Methods F, and B-E to give a colourless solid (330mg, 98.8%); IR (Solid) 3708.0, 3680.6, 2865.2, 1705.6, 1493.9, 1346.0, 1262.7, 1182.4, 1132.5, 1054.7, 1033.0, 1013.0, 703.8 cm^{-1}; ^1H NMR (400 MHz, d_6-DMSO δ 2.28-2.85 (5H, m), 4.05-5.20 (5H, m), 6.45 (1H, m), 6.95-7.32 (3H, m), 7.38-7.75 (6H, m), 8.30-8.85 (1H, m); ^{13}C NMR 100 MHz, d_6-DMSO) δ 25.66, 28.69, 34.93, (CH_2), 47.44, 51.84, 58.07, 72.77 (q, $CHCF_3$, J 120), 84.18 (d, CH_2F, J 176), 122.38, 125.17 (C), 126.55, 126.61, 127.89, 128.01, 128.07, 130.14 (CH), 136.68 (C), 171.34, 171.67, 172.01, 173.02, 202.55, 202.67, 202.97 (C=O); ^{19}F NMR (376MHz, d_6-DMSO) δ -74.21 (s), -226.62 (t, J 48), -226.99 (t, J 48), -232.67 (br m).

[3S/R, (2S,α-R)]-5-Fluoro-3-(1-(α-trifluoromethyl-(3-chloro benzyloxycarbonyl-1,2,3,4-tetrahydroquinolinyl-2-carboxamido)-4-oxo-pentanoic acid (Example 37)



This was prepared from (R)-(-)-a-(trifluoromethyl)-3-chlorobenzyl alcohol using procedures similar to those described above in Methods F, and B-E to give a colourless solid (323mg 99.1%); IR (solid) 3710.2, 3680.7, 2981.2, 2865.1, 1716.3, 1493.6, 1455.1, 1346.2, 1258.1, 1185.7, 1135.3, 1054.8, 1033.0, 1012.9 cm^{-1}; ^{1}H NMR (400 MHz, d_6-DMSO) δ 2.20-2.83 (5H, m), 3.65-5.22 (5H, m), 5.50 (1H, m), 6.90-7.30 (3H, m), 7.35-7.75 (5H, m), 8.25-8.90 (1H, m); ^{13}C NMR (100 MHz, d_6-DMSO) δ 25.64, 28.33,33.26, 34.93 (CH_2), 72.23 (q, $CHCF_3$, J 120), 84.14 (d, CH_2F, J 176), 122.15, 124.94 (C), 126.56, 126.62, 126.66, 127.93, 127.99, 130.18, 131.00 (CH), 133.64, 133.92 (C), 171.34, 171.68, 172.00, 173.13, 202.47, 202.62, 202.67, 202.97 (C=O); ^{19}F NMR (376 MHz, d_6-DMSO) δ -75.24 (s, CF_3), -226.66 (t, J 48), -227.00 (t, J 48), -232.39 (br m).δ

2-(1-Carbamoylmethyl-3-fluoro-2-oxo-propylcarbamoyl)-piperidine-1-carboxylic acid 3,4-dichloro-benzyl ester (Example 38)



This was prepared from [3S/R, (2S)]-3-(1-(3,4-Dichlorobenzyloxycarbonyl)-2-piperidinecarboxamido)-5-fluoro-4-oxo-pentanoic acid.

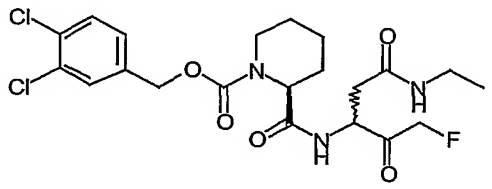
Method H

Compound 38

To a stirred solution of [3S/R, (2S)]-3-(1-(3,4-Dichlorobenzyloxycarbonyl)-2-piperidinecarboxamido)-5-fluoro-4-oxo-pentanoic acid (0.2g, 0.43 mmol) in tetrahydrofuran (THF)(2mL) 0°C was added N-(3-Dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride. A solution of ammonia in dioxane in THF (1.29 mmol) was added to the reaction mixture after it was allowed to warm up to ambient, and the solution was stirred overnight at room temperature. The reaction mixture was concentrated *in vacuo*. The residue was purified by flash chromatography to give the title compound as a colourless gum (mg): ^{1}H NMR (400MHz, $CDCl_3$) δ 1.3-1.8(5H, m, pip), 2.1-2.5(2H, m, Asp-CH2, pip), 2.6-3.2(2H, m, Asp-CH2, pip), 3.9-5.0(5H, m, Asp-CH, pip-CH, -CH2-F, NH), 5.0-5.2(2H, m, -CH2-Ar), 5.3(0.5H, m, NH), 6.6(0.4H, br s,

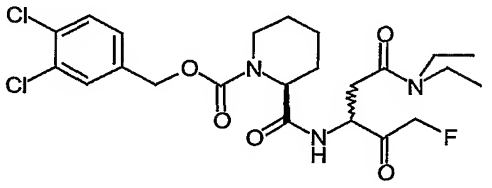
NH), 6.8-7.1(1H, m, NH), 7.2(1H, m, Ar), 7.5(2H, m, Ar); ^{19}F NMR (376MHz, CDCl3) δ -225.0, -225.8, -227.6, -227.9.

2-(1-Ethylcarbamoylmethyl-3-fluoro-2-oxo-propylcarbamoyl)-piperidine-1-carboxylic acid 3,4-dichloro-benzyl ester (Example 39)



This was prepared using procedures similar to those described above in Methods H, to give a colourless gum (mg): ^{1}H NMR (400MHz, $CDCl_3$) δ 1.2(3H, m, Et), 1.3-1.8(5H, m, pip), 2.1-2.5(2H, m, Asp-CH2, pip), 2.6-3.1(2H, m, Asp-CH2, pip), 3.1-3.5(2H, m, Et), 3.9-4.9(5H, m, Asp-CH, pip-CH, -CH2-F, NH), 5.0-5.2(2H, m, -CH2-Ar), 6.3-6.7(1H, m, NH), 7.2(1H, m, Ar), 7.4-7.5(2H, m, Ar) ^{19}F NMR (376MHz, CDCl3) δ -223.4, -226.6, -226.7.

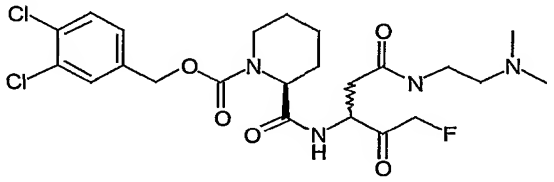
2-(1-Diethylcarbamoylmethyl-3-fluoro-2-oxo-propylcarbamoyl)-piperidine-1-carboxylic acid 3,4-dichloro-benzyl ester (Example 40)



This was prepared using procedures similar to those described above in Methods H, to give a colourless gum (mg): ^{1}H NMR (400MHz, $CDCl_3$) δ 1.0-1.3(6H, m, Et), 1.3-1.8(5H, m, pip), 2.2-2.3(1H, m, pip), 2.7-3.2(2H, m, Asp-

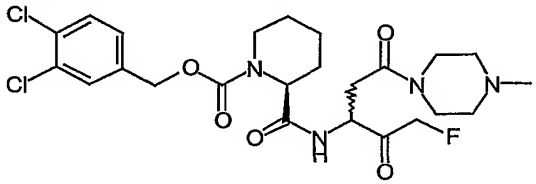
CH2), 3.2-3.4(4H, m, Et), 4.0-4.3(1H, m, pip), 4..7-5.4(6H, m, -CH2-F, Asp-CH, pip-CH, -CH2-Ar), 7.2(1H, m, Ar), 7.3-7.5(2H, m, Ar); ^{19}F NMR (376MHz, CDCl3) δ -232.3, -232.5, -232.9.

2-{1-[(2-Dimethylamino-ethylcarbamoyl)-methyl]-3-fluoro-2-oxo-propylcarbamoyl}-piperidine-1-carboxylic acid 3,4-dichloro-benzyl ester (Example 41)



This was prepared using procedures similar to those described above in Methods H, to give a colourless gum (mg): ^{1}H NMR (400MHz, CDCl3) δ 1.3-1.8(5H, m, pip), 2.1-2.4(9H, m, -CH2-CH2-N(Me)2), 2.6(1H, m, -CH2-CH2-N(Me)2), 2.7-3.1(3H, m, Asp-CH2, pip), 4.0-4.4(4H, m, -CH2-F, Asp-CH, pip), 4.6-4.7(1H, m, pip-CH), 4.8-4.9(1H, br s, NH), 5.0-5.2(2H, m, -CH2-Ar), 6.6-6.7(1H, m, NH), 7.2(1H, m, Ar), 7.5(2H, m, Ar); ^{19}F NMR (376 MHz, CDCl3) δ -222.4.

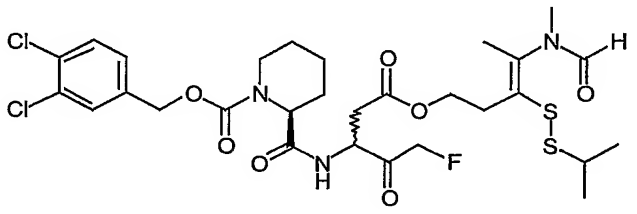
2-{3-Fluoro-1-[2-(4-methyl-piperazin-1-yl)-2-oxo-ethyl]-2-oxo-propylcarbamoyl}-piperidine-1-carboxylic acid 3,4-dichloro-benzyl ester (Example 42)



This was prepared using procedures similar to those described above in Methods H, to give a colourless gum

(mg): ^{1}H NMR (400MHz, $CDCl_3$) δ 1.3-1.9(5H, m, pip), 2.2-2.5(8H, m, pip), 2.7-3.2(2H, m, Asp-CH2), 3.2-3.3(1H, m, pip), 3.4-3.6(3H, m, pip), 3.6-3.7(1H, m, pip), 4.0-4.3(1H, m, pip), 4.7-5.4(6H, m, Asp-CH, pip-CH, -CH2-Ar, -CH2-F), 7.2(1H, m, Ar), 7.5(2H, m, Ar); ^{19}F NMR (376 MHz, CDCl3) δ -233.5, -233.6, -234.0.

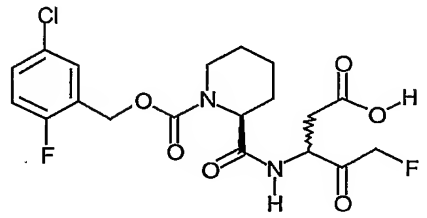
[3S/R, (2S)]-3-(1-(3,4-Dichlorobenzyloxycarbonyl)-2-piperidinecarboxamido)-5-fluoro-4-oxo-pentanoate, *N*-(4-hydroxy-2-isopropyl disulfanyl-1-methyl-butene)-*N*-methylformamide ester (Example 43)



This was prepared using procedures similar to those described above in Methods H from [3S/R, (2S)]-3-(1-(3,4-Dichlorobenzyloxycarbonyl)-2-piperidinecarboxamido)-5-fluoro-4-oxo-pentanoic acid and N-(4-hydroxy-2-isopropyl disulfanyl-1-methyl-butene) N-methylformamide (Int. J. Pharmaceutics, (1995), 116, 51) to give colourless gum (159 mg, 53%): ^{1}H NMR (400MHz, CDCl3) δ 1.1-1.8(11H, m, pip, iPr), 1.8-2.1(3H, m, N-Me), 2.2-2.3(1H, m, pip), 2.7-3.3(9H, m, iPr, -O-CH2-CH2-, Asp-CH2, pip), 4.0-4.3(3H, m, pip, -O-CH2-CH2-), 4.7-5.2(6H, m, -CH2-Ar, -CH2-F, pip, Asp), 6.9-7.1(1H, m, NH), 7.2-7.3(1H, m, Ar), 7.4-7.5(2H, m, Ar), 7.9-8.0(1H, br s, CHO); ^{19}F NMR (376MHz, CDCl3) δ -231.6, -231.7, -231.9.

[3S/R, (2S)]-3-(1-(5-Chloro-2-fluorobenzyloxycarbonyl)-2-piperidinecarboxamido)-5-fluoro-4-oxo-pentanoic acid

(Example 44)



This was prepared from 5-chloro-2-fluorobenzyl alcohol using procedures similar to those described above in Methods G, and B-E to give a white foam (7.5mg, 99% last step): IR (solid) 1788, 1670, 1491, 1424, 1404, 1250, 1178 cm-1cm^{-1}; ^{1}H NMR (400MHz, d_6-DMSO + TFA) 1.06-1.77 (5H, m), 1.95-2.20 (1H, m), 2.28-3.30 (3H, m), 3.75-4.00 (1H, m), 4.20-4.76 (2.5H, m), 4.95-5.35 (3.5H, m), 7.18-7.35 (1H, m), 7.37-7.60 (2H, m), 8.02-8.61 (1H, m); ^{13}C NMR (100MHz, d_6-DMSO) 19.94, 20.22, 24.43, 24.56, 27.08, 27.28, 27.65, 28.82, 32.87, 34.69, 42.16, 47.38, 47.52, 52.16, 52.38, 54.44, 54.52, 60.37, 60.55, 81.44, 81.51, 83.20, 83.26, 85.03, 103.94, 104.13, 117.47, 117.59, 117.70, 117.78, 126.18, 126.34, 128.57, 128.60, 129.99, 130.22, 155.19, 155.84, 158.01, 158.14, 158.52, 158.89, 160.47, 171.14, 171.68, 171.85, 172.08, 172.13, 173.31, 202.55, 202.68; ^{19}F NMR (376MHz, d_6-DMSO + TFA) δ-120.74, -120.85, -120.89, -120.96, -121.02, -226.68(t), -226.86(t), -226.95(t), -230.17(t), -230.44(t), -232.51(t), -232.58(t), -232.61(t), -232.64(t).

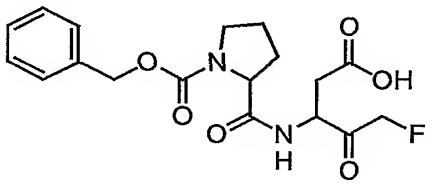
Enzyme Assays

The assays for caspase inhibition are based on the cleavage of a fluorogenic substrate by recombinant,

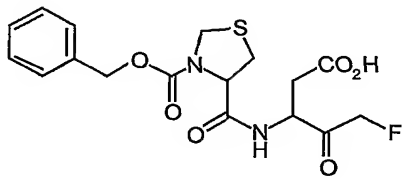
purified human Caspases -1, -3, or -8. The assays are run in essentially the same way as those reported by Garcia-Calvo et al. (*J. Biol. Chem.* **273** (1998), 32608-32613), using a substrate specific for each enzyme. The substrate for Caspase-1 is Acetyl-Tyr-Val-Ala-Asp-amino-4-methylcoumarin. The substrate for Caspases -3, and -8 is Acetyl-Asp-Glu-Val-Asp-amino-4-methylcoumarin.

The observed rate of enzyme inactivation at a particular inhibitor concentration, k_{obs}, is computed by direct fits of the data to the equation derived by Thornberry et al. (*Biochemistry* **33** (1994), 3943-3939) using a nonlinear least-squares analysis computer program (PRISM 2.0; GraphPad software). To obtain the second order rate constant, k_{inact}, k_{obs} values are plotted against their respective inhibitor concentrations and k_{inact} values are subsequently calculated by computerized linear regression.

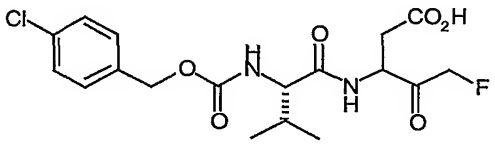
Table 2 shows a comparison between compounds of Examples 3 and 34 of this invention, and Cbz-Pro-Asp-fmk (WO 91/15557), Cbz-Thz-Asp-fmk (WO 99/477154) and 4-ClCbz-Val-Asp-fmk (WO 00/61542):



Cbz-Pro-Asp-fmk



Cbz-Thz-Asp-fmk



4-ClCbz-Val-Asp-fmk

Table 2. C-1, C-3, and C-8 Activity

No.	Kinact (x 1000 $M^{-1}s^{-1}$)		
	C-1	C-3	C-8
Example 3	318	239.5	122
Example 34	518	181	839
Cbz-Pro-Asp-fmk	7.5	41.5	15.5
Cbz-Thz-Asp-fmk	227.5	12.5	12
4-ClCbz-Val-Asp-fmk	69	50.5	175

As can be seen from the results in Table 2, the compounds of Example 3 and Example 34 have better activity than Cbz-Pro-Asp-fmk, Cbz-Thz-Asp-fmk and 4-ClCbz-Val-Asp across the range of caspases tested.

Inhibition of IL-1β secretion from Mixed Population of Peripheral Blood Mononuclear Cells (PBMC)

Processing of pre-IL-1β by caspase-1 can be measured in cell culture using a variety of cell sources. Human PBMC obtained from healthy donors provides a mixed population of lymphocyte and mononuclear cells that produce a spectrum of interleukins and cytokines in response to many classes of physiological stimulators.

Experimental procedure

The test compound is dissolved in Dimethyl Sulphoxide (DMSO,Sigma #D-2650) to give a 100 mM stock solution. This is diluted in complete medium consisting of RPMI containing 10% heat inactivated FCS (Gibco BRL

#10099-141), 2mM L-Glutamine (Sigma, #G-7513), 100U penicillin and 100 µg/ml streptomycin (Sigma #P-7539). The final concentration range of test compound is from 100 µM down to 6 nM over eight dilution steps. The highest concentration of test compound is equivalent to 0.1% DMSO in the assay.

Human PBMC are isolated from Buffy Coats obtained from the blood bank using centrifugation on Ficoll-Paque leukocyte separation medium (Amersham, #17-1440-02) and the cellular assay is performed in a sterile 96 well flat-bottomed plate (Nunc). Each well contains 100 µl of the cell suspension, 1 x 10^5 cells, 50 µl of compound dilutions and 50 µl of LPS (Sigma #L-3012) at 50 ng/ml final concentration. Controls consist of cells +/- LPS stimulation and a serial dilution of DMSO diluted in the same way as compound. The plates are incubated for 16-18h at 37°C in 5% CO_2 & 95% humidity atmosphere.

After 16-18 h the supernatants are harvested after centrifuging the plates at 100 x g at 18°C for 15 min and assayed for their IL-1β content. Measurement of mature IL-1β in the supernatant is performed using the Quantikine kits (R&D Systems) according to manufacturer's instructions. Mature IL-1β levels of about 600-1500 pg/ml are observed for PBMCs in positive control wells.

The inhibitory potency of the compounds can be represented by an IC_{50} value, which is the concentration of inhibitor at which 50% of the mature IL-1β is detected in the supernatant as compared to the positive controls. Table 3 shows the inhibition of IL-1β secretion from PBMC for the compounds of Example 3 and Example 5 and the known Cbz-Pro-Asp-fmk and Cbz-Thz-Asp-fmk.

Table 3. Inhibition of IL-1β secretion from PBMC

No.	IC_{50} (nM)
Example **3**	1150
Example **5**	500
Cbz-Pro-Asp-fmk	>10000
Cbz-Thz-Asp-fmk	2308

As can be seen from the results in Table 3, the compounds of Example 3 and Example 5 provide much better inhibition of IL-1β secretion from PBMC than does Cbz-Pro-Asp-fmk or Cbz-Thz-Asp-fmk.

Hypoxia-induced apoptosis of cortical neurons assay

Caspases have been shown to significantly contribute to neuronal cell damage in a number of neurological disorders (Drug News Persp., (2000), **13(1)**, 5-11). Apoptosis can be induced by growth factor withdrawal and by hypoxia. This assay measures the extent of DNA fragmentation indicating the effectiveness of caspase inhibitors to prevent apoptosis.

Experimental procedure

Cortical neurons were dissociated from Wistar rat embryos (E17) by a modification of the procedure of Rogers et al 1997, Brain Res Bulletin 44:131. Cerebral cortices were isolated aseptically from 15-20 Wistar rat embryos. A cell suspension was prepared by mincing the cerebral cortices and digesting them with papain. Cells were

washed with ovomucoid enzyme inhibitor and DNaseI and plated onto Poly-D lysine coated plates in high glucose DMEM containing 10% heat-inactivated foetal calf serum, L-glutamine, penicillin and streptomycin. The yield of neurons was10^7 per embryo and they were 80-90% viable as assessed by Trypan blue exclusion.

Cells were seeded at $1x10^6$ cells per cm^2 in 96-well plates and cultured in complete medium (in high glucose DMEM containing 10% heat-inactivated foetal calf serum, L-glutamine, penicillin and streptomycin) at 37°C in a normal atmosphere for 48 hours prior to the hypoxia experiments. Hypoxia was performed as described (Tamatani et al.1998, Molecular Brain Research, 58:27). The normal cell medium was replaced by hypoxic medium and cells were incubated in an atmosphere of 95% N_2/ 5% CO_2 for 42 hours. For compound testing, compounds were dissolved in DMSO at 100mM then diluted in medium and added to the culture at the beginning of the hypoxic period. Apoptosis was measured using an ELISA assay to detect DNA fragmentation (Roche). Controls included cells cultured in aerobic conditions in serum-containing medium. Table 4 shows the activity of the compounds of Example 34 and Example 23 and the known Cbz-Pro-Asp-fmk, Cbz-Thz-Asp-fmk and 4-ClCbz-Val-Asp-fmk in the hypoxia induced apoptosis of cortical neurons assay .

Table 4. Activity in the hypoxia induced apoptosis of cortical neurons assay

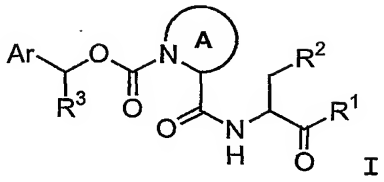
No.	IC_{50} (nM)
Example **34**	463
Example **23**	336
Cbz-Pro-Asp-fmk	2776
Cbz-Thz-Asp-fmk	1563
4-ClCbz-Val-Asp-fmk	1983

As can be seen from the results in Table 4, the compounds of Example 34 and Example 23 are much more potent than Cbz-Pro-Asp-fmk, Cbz-Thz-Asp-fmk and 4-ClCbz-Val-Asp-fmk in the hypoxia induced apoptosis of cortical neurons assay.

While we have described a number of embodiments of this invention, it is apparent that our basic examples may be altered to provide other embodiments, which utilize the compounds and methods of this invention. Therefore, it will be appreciated that the scope of this invention is to be defined by the appended claims rather than by the specific embodiments, which have been represented by way of example.

What is claimed is:

1. A compound of formula I:



I

wherein:

Ring A is an optionally substituted piperidine, tetrahydroquinoline or tetrahydroisoquinoline ring;

R^1 is hydrogen, CHN_2, R, or $-CH_2Y$;

R is an optionally substituted group selected from an aliphatic group, an aryl group, an aralkyl group, a heterocyclic group, or an heterocyclylalkyl group;

Y is an electronegative leaving group;

R^2 is CO_2H, CH_2CO_2H, or esters, amides or isosteres thereof;

Ar is an optionally substituted aryl group; and

R^3 is hydrogen, an optionally substituted C_{1-6} alkyl, F_2, CN, aryl or R^3 is attached to Ar to form an unsaturated or partially saturated five or six membered fused ring having 0-2 heteroatoms.

2. The compound of claim 1 having one or more of the following features:

(a) R^1 is CH_2F;

(b) R^2 is CO_2H or esters, amides or isosteres thereof;

(c) R^3 is hydrogen or an optionally substituted C_{1-6} alkyl; and

(d) Ar is an optionally substituted aryl.

3. The compound of claim 2 having the following features: (a) R^1 is CH_2F; (b) R^2 is CO_2H or esters, amides or isosteres thereof; (c) R^3 is hydrogen or an optionally substituted C_{1-6} alkyl; and (d) Ar is an optionally substituted aryl.

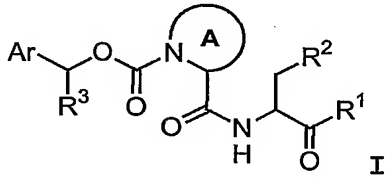
4. The compound of claim 3 where Ring A is a piperidine ring.

5. The compound of claim 3 where Ring A is a tetrahydroquinoline ring.

6. The compound of claim 3 where Ring A is a tetrahydroisoquinoline ring.

7. The compound of claim 1, wherein the compound is selected from the compounds listed in Table 1.

8. A method for treating a condition or disease state in mammals that is alleviated by treatment with a caspase inhibitor, comprising administering to a mammal in need of such a treatment a therapeutically effective amount of a compound of formula **I**:



I

wherein:

Ring A is an optionally substituted piperidine, tetrahydroquinoline or tetrahydroisoquinoline ring;

R^1 is hydrogen, CHN_2, R, or $-CH_2Y$;

R is an optionally substituted group selected from an aliphatic group, an aryl group, an aralkyl group, a heterocyclic group, or an heterocyclylalkyl group;
Y is an electronegative leaving group;
R^2 is CO_2H, CH_2CO_2H, or esters, amides or isosteres thereof;
Ar is an optionally substituted aryl group; and
R^3 is hydrogen, an optionally substituted C_{1-6} alkyl, F_2, CN, aryl, or R^3 is attached to Ar to form an unsaturated or partially saturated five or six membered fused ring having 0-2 heteroatoms.

9. The method of claim 8 wherein the compound has one or more of the following features: (a) R^1 is CH_2F; (b) R^2 is CO_2H or esters, amides or isosteres thereof; (c) R^3 is hydrogen or an optionally substituted C_{1-6} alkyl; and (d) Ar is an optionally substituted aryl.

10. The method of claim 9 wherein the compound has the following features: (a) R^1 is CH_2F; (b) R^2 is CO_2H or esters, amides or isosteres thereof; (c) R^3 is hydrogen, CF_3 or C_2F_5; and (d) Ar is an optionally substituted aryl.

11. The method of claim 8 wherein the compound is selected from the compounds listed in Table 1.

12. The method of claim 8 wherein the disease is selected from an IL-1 mediated disease, an apoptosis mediated disease, an inflammatory disease, an autoimmune disease, a destructive bone disorder, a proliferative disorder, an infectious disease, a degenerative disease, a disease associated with cell death, an excess dietary

alcohol intake disease, a viral mediated disease, uveitis, inflammatory peritonitis, osteoarthritis, pancreatitis, asthma, adult respiratory distress syndrome, glomerulonephritis, rheumatoid arthritis, systemic lupus erythematosus, scleroderma, chronic thyroiditis, Grave's disease, autoimmune gastritis, diabetes, autoimmune hemolytic anemia, autoimmune neutropenia, thrombocytopenia, chronic active hepatitis, myasthenia gravis, inflammatory bowel disease, Crohn's disease, psoriasis, atopic dermatitis, scarring, graft vs host disease, organ transplant rejection, osteoporosis, leukemias and related disorders, myelodysplastic syndrome, multiple myeloma-related bone disorder, acute myelogenous leukemia, chronic myelogenous leukemia, metastatic melanoma, Kaposi's sarcoma, multiple myeloma, haemorrhagic shock, sepsis, septic shock, burns, Shigellosis, Alzheimer's disease, Parkinson's disease, Huntington's disease, Kennedy's disease, prion disease, cerebral ischemia,epilepsy, myocardial ischemia, acute and chronic heart disease, myocardial infarction, congestive heart failure, atherosclerosis, coronary artery bypass graft, spinal muscular atrophy, amyotrophic lateral sclerosis, multiple sclerosis, HIV-related encephalitis, aging, alopecia, neurological damage due to stroke, ulcerative colitis, traumatic brain injury, spinal cord injury, hepatitis-B, hepatitis-C, hepatitis-G, yellow fever, dengue fever, or Japanese encephalitis, various forms of liver disease including alcoholic hepatitis, renal disease, polyaptic kidney disease, H. pylori-associated gastric and duodenal ulcer disease, HIV infection, tuberculosis, and meningitis.

13. The method of claim 8 wherein the compound is used to treat complications associated with coronary artery bypass grafts.

14. The method of claim 8 wherein the compound is used for the preservation of cells, said method comprising the step of bathing the cells in a solution of the compound or a pharmaceutically acceptable derivative thereof.

15. The method of claim 8 wherein the compound or a pharmaceutically acceptable derivative thereof is used for an organ transplant or for preserving blood products.

16. The method of claim 8 wherein the compound is used as a component of immunotherapy for the treatment of cancer.

17. A pharmaceutical composition comprising a compound according to any of claims 1-7 and a pharmaceutically acceptable carrier.

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(74) Agents: **SILVERMAN, Ian**; Vertex Pharmaceuticals Inc., 130 Waverly Street, Cambridge, MA 02139-4242 et al. (US).

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Published:
— *with international search report*

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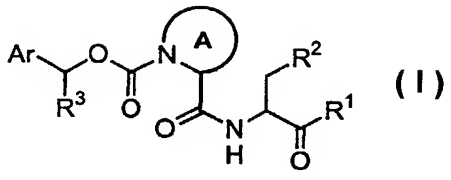
For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: CASPASE INHIBITORS AND USES THEREOF



(I)

(57) Abstract: This invention provides caspase inhibitors having the formula: (I) wherein Ring A is an optionally substituted piperidine, tetrahydroquinoline or tetrahydroisoquinoline ring; R^1 is hydrogen, CHN_2, R, or $-CH_2Y$; R is an optionally substituted group selected from an aliphatic group, an aryl group, an aralkyl group, a heterocyclic group, or an heterocyclylalkyl group; Y is an electronegative leaving group; R^2 is CO_2H, CH_2CO_2H, or esters, amides or isosteres thereof; Ar is an optionally substituted aryl group; and R^3 is hydrogen, an optionally substituted C_{1-6} alkyl, F_2, CN, aryl or R^3 is attached to Ar to form an unsaturated or partially saturated five or six membered fused ring having 0-2 heteroatoms. The compounds are useful for treating caspase-mediated diseases in mammals.

WO 01/90070 A3

INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/US 01/17075

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D211/60 C07D215/48 C07D401/12 C07D409/12 A61K31/4545 A61P37/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 99 47154 A (CYTOVIA INC) 23 September 1999 (1999-09-23) cited in the application abstract page 10, line 24 ---	1,8,17
A	WO 99 18781 A (CYTOVIA INC) 22 April 1999 (1999-04-22) cited in the application abstract Compound 5 page 12 --- -/--	1,8,17

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 January 2002	15/01/2002

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/US 01/17075

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WO 91 15577 A (IMMUNEX CORP) 17 October 1991 (1991-10-17) cited in the application abstract Cbz-Pro-Asp-CH2F page 30, line 32	1,8,17

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